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ANTHRACNOSE OF LETTUCE CAUSED BY MARSSONINA PANATTONIANA

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INTRODUCTION

Lettuce (*Lactuca sativa*) is not an easy crop to grow successfully, because of the peculiarities of the plant, its quick response to unfavorable conditions, and its susceptibility to disease. Any disfigurement of the leaves renders it unmarketable. In this regard it is rather unique, for most other food plants may be attacked in some part by insects or fungi, and still may not be a total loss.

The disease considered in this paper does its chief damage to greenhouse lettuce. The methods of culture under glass are very important factors in determining the extent of damage from this disease. In general, several crops are grown during the winter season. Seeds are sown very thickly in beds, usually in raised benches in a greenhouse devoted to the production of seedlings. After one or more transplantings the small plants, 3 or 4 inches tall, are set out with 6-inch spacings in beds in the main greenhouse. The soil is heavily enriched with manure or other fertilizer. The watering is done either with hose or, in the better greenhouses, with an overhead sprinkling system. The temperature is kept about 55° F.; and with the best growers ventilation is maintained practically all of the time, excepting, of course, the extremely cold nights.

Seed beds are planted so that a succession of crops can be raised. Although some head lettuce is grown, by far the bulk of the crop is of the leaf-lettuce type. The crop is harvested when the plants weigh from 3 to 6 ounces, the state of the market and the maturity of the lettuce being governing factors. The bunches are trimmed as harvested, the débris being turned under as a general rule.

Naturally with such intensive methods as the heavy investment in glass and equipment demands, many parasitic diseases become im-

[1] This work was done at the suggestion of and along lines outlined by Dr. G. H. Coons, of the Michigan Agricultural College, and I am indebted to him for help in the preparation of the manuscript. I also wish to thank Dr. E. A. Bessey, Professor of Botany in the Michigan Agricultural College, for kindly assistance and encouragement during the course of the work.

portant. The general effects of these troubles may be contrasted with the anthracnose. Aside from the insect and nematode depredations, we have in general two types of injury to the mature plants; diseases which rot the plants more or less, and those which disfigure the leaves. In the first group may be mentioned drop, caused by *Sclerotinia libertiana*, and graymold, caused by *Botrytis* sp. Occasionally the softrot caused by *Bacillus carotovorus* causes damage. Frequently lesions are found in the petioles and blade which lead to the rotting off of the other leaves of the plant. This disease is called "black stemrot," or "bottom-rot," and species of Rhizoctonia have been found associated with this trouble.

Of the leaf diseases, the downy-mildew, caused by *Bremia lactuca*, and the anthracnose are the most important. The effects of *B. lactuca* are such as would be expected from one of the downy-mildews. Light-colored areas are noted on the upper surface of the leaves, while the lower surface shows the conspicuous tufts of conidiospores of the organism. With young plants a drying up of the leaves may occur.

COMMON NAME OF THE DISEASE

In the previous accounts of this disease (*3*),[1] several common names have been used: "anthracnose," "shothole," "leaf-perforation," and "rust." In Michigan greenhouses the name "rust" is probably most common. The causal organism, however, has no connection with the true rusts of plants. Hence, this name, though fairly descriptive and popular, is, according to the prevailing usage among pathologists, not to be recommended. The names "shothole" and "leaf-perforation," while very appropriate, in so far as the lesions on the leaf blade are concerned, do not properly consider the effect on the midrib, which is by far the more disfiguring phase of the trouble. The name least open to objection, and the one which is in accord with the best usage among plant pathologists, is "anthracnose." This term has become a common word among growers of other crops affected by similar parasitic diseases, and will no doubt be readily accepted by greenhouse men.

PREVIOUS INVESTIGATIONS

The first account of the anthracnose of lettuce and the associated organism was given by Berlese (*2*) in 1895. This writer, in a short note after a description of the signs of the malady, named the associated organism "*Marssonia panattoniana*." As will be seen in the discussion of the etiology of this disease, Berlese recognized the parasitic nature of the fungus, but did not make conclusive inoculation experiments.

At almost the same time Selby (*8, p. 224*) published a brief note in which he described the appearance of the affected plants. The associated fungus was named *Marssonia perforans* E. and E., Ellis and Ever-

[1] Reference is made by number (italic) to "Literature cited," p. 280.

hart having determined the fungus from Selby's collections. The fungus was reported as being quite prevalent in Ohio greenhouses that year and as causing considerable loss to lettuce growers. In a succeeding publication Selby (*9*) repeated the statements of former bulletins and stated that the fungus had since been discovered at other points in the United States.

No doubt the most important contribution to the literature of this disease was that of Appel and Laibach in 1908 (*1*). In the Province of Brandenburg, Germany, an epidemic caused by the fungus called *Marssonia panattoniana* in the spring of 1907 caused many growers to destroy thousands of plants. It is noteworthy that the fungus appeared in the open, while American observations dealt with the disease in greenhouse plants. Appel and Laibach made important notes on the morphology of the fungus, grew it in pure culture, and made inoculation experiments with spores from the diseased host.

Dandeno in 1907 (*4*) from examinations of Michigan material came to the conclusion that the fungus, called by him "*Marssonia perforans*," was not properly classified as Marssonia, but was rather a species of Didymaria.

Kirchner, in 1906 (*6, p. 376, 389*), without citing his authority, records the disease from Holland and Italy on endive, as well as lettuce.

Aside from repetition of statements from former literature in the general handbooks of plant diseases, no other publication on this disease or its causal organism has come to the writer's attention.

DISTRIBUTION OF THE FUNGUS

The distribution of the fungus is no doubt wide. No record exists of its presence in Europe, except in Germany, Holland, and Italy. Meagerness of accounts may be responsible for this lack of record. From the notes in the literature, the fungus is known from Ohio and Michigan. The Ohio records date from 1895. The first Michigan specimens were collected on March 20, 1897, at Grand Rapids, by B. O. Longyear. Since then the fungus has been found repeatedly at various parts of the State. During an excessively wet June in 1916 it was found at East Lansing as a serious pest on lettuce grown in the garden.[1] Early in 1917 it was observed by the writer in a greenhouse at Ithaca, N. Y., where 5 to 10 per cent of the plants were a total loss, and many of the remainder were so badly affected as to require the stripping of the outer leaves. Since that time it has been reported from various parts of New York State by Mr. H. W. Dye, Dr. Charles Chupp, and Dr. I. C. Jagger. Dr. Jagger informed the writer that he had noticed the disease in previous years in New York State.

[1] The writer is indebted to Dr. G. H. Coons for this report.

The records of the Plant Disease Survey of the United States Department of Agriculture are as follows:

No date. Michigan (R. H. Pettit).[1]
Caused loss of thousands of dollars in the forcing houses.
1910. Ohio (A. D. Selby).
Reported in one county.
1912. Oregon (H. S. Jackson).
Limited.
Utah (C. N. Jensen).
15 per cent in some greenhouses.
1915. North Carolina (H. R. Fulton).
Loss considerable.
Washington (F. D. Heald and D. C. George).
Occurrence only.

ECONOMIC IMPORTANCE OF ANTHRACNOSE

The disease was stated by Selby (*8*) to be very important in 1895. It is reported to have been quite prevalent in Ohio during that year and to have caused considerable losses to the lettuce growers.

Appel and Laibach (*1*) reported a 50 to 60 per cent loss in the fields about Brandenburg, Germany.

Authoritative reports from growers in Grand Rapids in 1898 and a few years following indicate that the disease was serious enough to cause the loss of most of the crop in practically the entire acreage about Grand Rapids. Since that time the losses have been less uniform, and at the present time only occasional greenhouses show serious outbreaks of the disease. Careful search in almost any greenhouse will reveal occasional plants, but few epidemic outbursts of the disease are now reported.

A plausible explanation of this decrease in the amount of disease is given under the discussion of dissemination of the fungus.

SYMPTOMS OF THE DISEASE

The general effect on the plant in this anthracnose is to produce a general dwarfing and discoloration (see Pl. C). Beds with affected plants have a brown or yellowish appearance. In the field the disease in a moist season may lead to death of the outer leaves. Since the attack is progressive, passing from older to younger inner leaves, the effect is to make plants small and of second grade, if not indeed a total loss. The fungus is found on the leaf blades and on the midribs.

ON THE LEAF.—The fungus produces a characteristic spot on the leaves of affected lettuce plants. The diseased areas show first as small, pinpoint, water-soaked spots. These rapidly enlarge, and soon a straw-colored spot 3 to 4 mm. in diameter is produced (Pl. 20, A). The spot is either circular or angular, depending on the proximity of large veins.

[1] February, 1906 (from records of the Michigan Experiment Station). At this time research by Prof. Pettit and Mr. Moses Craig resulted in recommendations of subirrigation as means of water supply, together with surface applications of sand. No publication was made of work done.

In the last stages the spots show dead areas at the center, and these may fray or drop out, giving the leaf a characteristic shot-hole appearance. Such a leaf appears as if gnawed by insects. Affected leaves wither quickly, and the lettuce is liable to rot under storage conditions. Evidence of spore production is seldom seen with the unaided eye even in cases of severe infection, although spores can always be obtained from such spots. If, however, the diseased leaves are placed under moist conditions, a film of pinkish-white spores appears at the centers of the diseased spots.

ON THE MIDRIB.—The lesion on the midrib is of the type characteristic of the anthracnoses. Sunken elliptical spots commonly appear in great numbers (Pl. 20, B). These spots vary with the age and the condition of the leaf. They are elongated in the direction of the long axis of the leaf, 4 to 5 by 2 to 2.5 mm., and are not noticeably sunken. This elongation of the spots on the nerve is undoubtedly due to the shape of the cells in that situation, which are very long and narrow, and offer less resistance to the progress of the parasite in a longitudinal than in a transverse direction, on account of the fewer cross walls. The depression is more apparent in these elongated nerve spots than in the round spots on the blade of the leaf, because the tissue of the nerve is thicker and more fleshy, and the collapse of a greater number of cells is possible.

These spots begin as barely noticeable water-soaked areas. After a few days the spots become a straw-yellow, while in age they are reddish yellow. Badly affected midribs present a very irregular contour. With the progress of the disease the functions of the entire leaf are disturbed. Occasionally the attack is severe enough and the lesions deep enough for the weight of the leaf to cause the breaking or bending at the affected point. Badly affected leaves often wither, owing, no doubt, to the loss of water from the broken epidermis.

ETIOLOGY OF THE DISEASE

Since Berlese's first report (*2*), the etiological relation of *Marssonina panattoniana* (*Marssonia perforans* in American literature) to the disease has been assumed. Berlese, however, performed no experiments to prove the causal relation. The work of Selby was of similar character. Appel and Laibach cultured the organism, but gave no evidence in their article of having used pure cultures in inoculation experiments. The successful inoculation experiments which they described were those in which spores from diseased plants were transferred to healthy plants.

Proof, therefore, of the etiology of the anthracnose of lettuce depends entirely upon constant association of the host and organism along with evidence of the infectious nature of the disease. Although this is no doubt sufficient in the case of the organisms of such known pathogenic habit as the Melanconiales, it is now possible to complete the proof.

Diseased lettuce leaves were collected from a greenhouse at Grand Rapids on January 16, 1915. Some of the material was placed in moist chambers where it remained until February 20. A microscopic examination showed an abundance of typical spores. A large drop of water was placed on the spot. After a short time this water was drawn up into a capillary tube. Plates of prune-juice agar were planted by making streaks across them with the capillary tube. Fungus colonies appeared in abundance and were transferred to test tubes. After two days, sporulation which was recognized as typical had occurred, and plates were poured by the loop dilution method, with prune-juice agar. A single spore was located with a microscope, and a pure culture was thus obtained. This was increased, and the subcultures were used for inoculation. Twelve lettuce plants growing in 6-inch pots in the greenhouse were inoculated by means of punctures along the upper surface of the midribs. Control plants were punctured with a sterile needle. In five days typical lesions of the disease had appeared on the inoculated plants, and the organism was reisolated by the loop dilution method, the spores being lifted from an acervulus by means of a sterile platinum spatula. None of the control plants became diseased. A new single spore culture was thus obtained whose relation to the disease had been established by isolation, inoculation, and reisolation.

In other experiments with pure cultures, typical lesions have been produced by placing material on the uninjured surface of the leaf blades and midribs (Pl. 20, C).

During the course of the work attempts to inoculate species closely related to *Lactuca sativa*—namely, *L. floridana*, *L. scariola*, and *Cichorium intybus*—failed; but inoculations of all varieties of *L. sativa* tested—namely, Black-Seeded Tennis Ball, Prize Head, and Grand Rapids Forcing—were found to be uniformly successful.

INFECTION PHENOMENA

Experiments started on April 1, in which spores from recently isolated cultures were sown on cover glasses which had been smeared with a thin glucose agar and which were subsequently placed over Van Tieghem rings, proved that the time necessary for germination is very short, ranging from four to eight hours, with an average of six hours at room temperature (25° C.). This experiment was continued in order to determine the time necessary for spore formation. This period was found to be very short also, spores being produced from newly formed conidiophores 30 hours after germination of the original spores and being present in great quantities at the end of 40 hours. On April 18, spores from a fresh culture were sown on the leaf of a young lettuce plant, and the pot containing it was placed beside a compound microscope so that the inoculated leaf could be held firmly on the stage. A thin cover glass

was placed over the leaf where the spores were sown, and the microscope focused upon the surface of the leaf. The spores being hyalin were somewhat difficult to make out with the cells of the leaf as a background;

FIG. 1.—*Marssonina panattoniana* (×300): *A*, Germination of spores; *B*, the same spores two hours later; *C*, after four hours; *D*, after six hours; E, after eight hours.

but with a proper adjustment of the diaphragm and condenser, this was accomplished. By this method it was determined that the required period for germination on the host plant is approximately the same as

when grown in culture (about six hours). The germ tube of some of the spores could be seen to enter the cell walls of the epidermal cells. Penetration took place at a point of contact of the epidermal cells; hence, the germ tube probably grew between the cells throughout. In no case was the germ tube observed within a cell (fig. 1, D, showing penetration). In order to determine whether penetration had actually taken place, water was introduced under the cover glass by means of a capillary tube. In the slight currents which were set up, the free ends of the spores whose germ tubes had penetrated swayed back and forth, but did not wash away, as did those whose germ tubes had not entered the host tissue.

RELATION OF TEMPERATURE TO INFECTION

As the season progressed it was found increasingly difficult to infect plants in the greenhouse, and after June 1 this was quite impossible. The assumption that temperature had considerable influence on the ability of the fungus to infect healthy plants was proved by the following experiment.

Twenty-five plants were inoculated from a 22-day-old culture, by smearing mycelium on the upper and lower surfaces of the leaves. Ten plants were left uncovered in the greenhouse, and 10 were covered with bell jars. The temperature of the greenhouse varied from 25° to 38° C. Five plants were placed in a low-temperature thermostat at 15°. The air conditions in the thermostat were humid, being comparable to the plants under the bell jars.

After three days the plants were examined. Those in the greenhouse were apparently unaffected, but the plants which had been placed in the refrigerator had developed the characteristic lesions of *M. panattoniana*. The greenhouse plants were subsequently watched for 16 days, but no infection could be observed. Evidently temperature is an important factor in the inception of this disease. These results indicate that infection periods occur in cool weather rather than in hot, bright weather. This is in accordance with the observations of Appel and Laibach (*1*), who say:

> . . . It may happen in warm dry weather that the attacked leaves fall and yet the plants in spite of that fact go on to the formation of usable lettuce. Damp weather is especially favorable to the development of the fungus, and therefore it is clear that due to this cold wet spring (1907) the disease has taken on such a threatening character.—TRANSLATION.

RELATION OF MOISTURE TO DEVELOPMENT OF LESIONS

Twenty plants were inoculated and placed in a Wardian case equipped with a fine jet for water adjusted to form a spray. A tested[1] hair hygrometer registered 70 per cent. The saturated plants were allowed to remain

[1] Compared with sling psychrometer.

for a week, after which they were removed. The spray was then cut off, and after normal greenhouse conditions had been restored, another set of 20 plants was inoculated and placed in the case for the same length of time. The ordinary greenhouse temperature (65° to 75° F.) prevailed during the entire experiment. No appreciable difference could be observed in the rapidity with which the fungus spread through the leaf in the two cases compared. Apparently, once the infection occurs, it is quite as easy for this fungus to spread within the host under ordinary conditions as under humid conditions. Aside, therefore, from the relation of water to dissemination and germination, it is evident that temperature plays a more important rôle than the moisture relations of the host.

DISSEMINATION OF THE DISEASE

The source of this trouble in greenhouses is somewhat uncertain. The disease was noticed in 1916 by Dr. G. H. Coons in an isolated planting of lettuce on new ground which had never borne a crop before. It has occurred suddenly in greenhouses where no previous outbreaks were known. This indicates the possibility of seed transference of the fungus, or else the existence of the fungus as a soil saprophyte. The experiments reported under "Physiological relations of the causal organism" showed that the spores were not able to survive drying on glass for five days. They might, however, be more resistant in the mucilaginous coat of lettuce seeds. The fungus might also be transported in bits of trash carried with the seed. The presence of the fungus in the seed or in the soil has not as yet been demonstrated.

Natural infection proceeds from the outer, lower leaves to the inner ones, so there is good ground for assuming that the common source of infection in greenhouses is the soil, or rather diseased trash, which is left to overwinter in the ground. Additional evidence to support this view is that diseased leaves which are exposed in the field, packed in sand in cans from January 20 until April 1, were found to contain viable spores. The fungus spreads to the inner leaves either by local infection or by the actual penetration of the mycelium. Upon stripping a leaf from the head it can be seen that the newly infected areas of the inner leaves are adjacent to the old spots on the outer leaves. The disease thus spreads step by step inward until the whole plant is affected. Spots high up on the leaves come from infection by spores. A careful examination of leaves at the early stage of infection reveals minute discolored spots at a considerable distance from diseased areas. By the aid of the microscope a spore can usually be found on the surface of these minute spots, with its germ tube boring into the tissues between the cells. During a dashing rain these spores are possibly splashed high up on the plant from old spots which have begun to sporulate; or they may be carried by some of the many insects which infest the lettuce plant. The fact before mentioned, that the spots are more numerous in the lower part of

the leaf, may be explained by the tendency of the rain water to run down and accumulate in the axils of the leaves, washing the spores with it.

The appearance and location of the newly infected spots on the leaves led to the belief that the dashing rains in the field and the use of hose for watering or syringing in the greenhouses were accountable for the spread of the disease from plant to plant. The following experiment was performed to determine the relation of the splashing of water from plant to plant to the dissemination of the fungus.

Two plots of lettuce in a bench were separated by a wooden partition covered with tar paper, and the rows parallel to and nearest the walk were inoculated with *M. panattoniana* in wounds made with a sterile

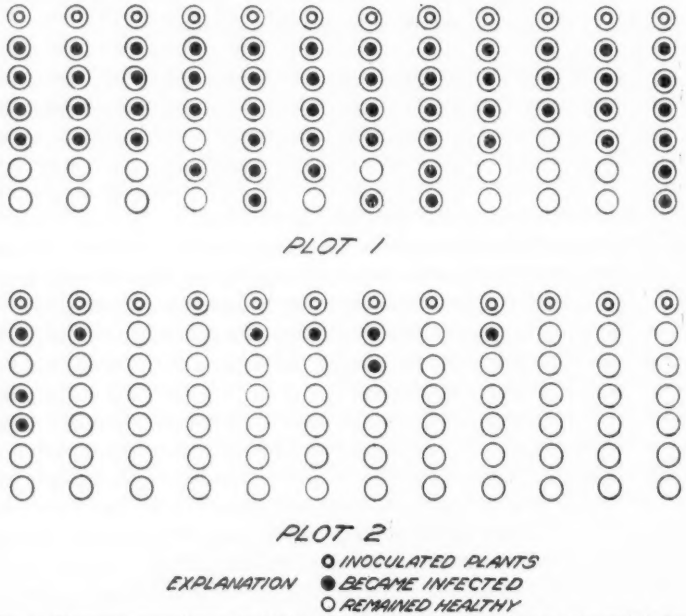

FIG. 2.—Diagrams representing results (plot 1) of watering with the hose and (plot 2) by subirrigation.

spatula. Plot 1 was watered by spraying with the hose. In plot 2 a trench was dug between each row, transverse with the length of the bench, and covered with a long flat board. A notch was cut in each of these boards at the end nearest the walk, and the plants were watered by inserting the hose through this notch and filling the trench with water. The surface of the ground and the plants above ground were thus kept dry during the watering process.

In the plot which was watered by spraying with a hose 55 of the 72 plants were infected in one week. In the plot watered by subirrigation only 9 plants out of the total 72 had been infected in the same period. The results of this experiment are shown very clearly in the accompanying diagrams (fig. 2). The objection may be raised that it was the wet-

ness of the leaves and not the splashing that was responsible for the spread of the disease in this case. Undoubtedly a wet leaf is a more suitable medium for most spores than a dry one, but the wetness of the leaf is of no avail when the mechanism for bringing a spore to it is lacking. It may be mentioned here that in greenhouses, where water drips upon diseased and healthy plants, it has been observed that the plants in the vicinity of the diseased plant become infected, owing to the splashing of drops of water containing spores from diseased to healthy leaves. When water drips on healthy plants alone, the leaves are equally wet, but no infection occurs, indicating that wetness of the leaves alone is not responsible. It is clear that the splashing of water contributes quite largely to the spread of the pathogene in the greenhouse, and probably rain is responsible to a large extent for its spread in the fields. The relation of this to the control of the disease will be considered later.

NAME OF THE PATHOGENE

The organism seems to be properly placed among the Melanconiales. In America the fungus is commonly called "*Marssonia perforans*." The description of this organism fits very well the Michigan specimens. However, Berlese (*2*) described what is undoubtedly the same organism several months previous to the American description. Accordingly the specific name "*panattoniana*" has priority. This was recognized by Appel and Laibach (*1*) who retained the name "*Marssonia panattoniana*."

Mangus (7) has shown that the genus Marssonia (sometimes erroneously spelled "Marsonia") was given in 1861 by Karsten to a Phanerogamic genus. This antedates Marssonia Fischer as a genus of fungi by 13 years. Magnus accordingly has proposed the name "Marssonina" for the preempted genus, and has made the new combination *Marssonina panattoniana* (Berlese) Magnus.

The synonyms of this fungus are as follows:

Marssonina panattoniana (Berlese) Magnus

Marsonia panattoniana Berlese, 1895, in Riv. Pat. Veg., v. 3, no. 5/12, p. 342
Marsonia perforans Ell. and Ev., 1896, in Ohio Agr. Exp. Sta. Bul. 73, p. 225.
Didymaria perforans (Ell. and Ev.) Dandeno, 1906, *in* 8th Rpt. Mich. Acad. Sci., p. 47.
Marssonina panattoniana Magnus, 1906, *in* Hedivigia, Bd. 45, Heft 2, p. 88–91.

MORPHOLOGY OF THE CAUSAL ORGANISM

The fungus which causes the anthracnose of lettuce is a typical member of the order Melanconiales. The mycelium of the fungus is composed of slender, hyalin septate threads which penetrate between the cells of the host. This mycelium is very scanty within the diseased spots except as it becomes matted to form a fruiting layer. This fruiting layer is formed beneath and upon the epidermis at a lesion. The fruiting layer is much more diffuse than that of Gloeosporium or Colletotrichum. For this reason Dandeno (*4*) has suggested that the fungus is in reality a species of Didymaria.

Sections through a lesion in which abundant sporulation occurs show acervuli consisting of closely packed, upright conidiophores, 20 to 60 μ tall (fig. 3, E). From the apexes of these conidiophores slightly curved, 1-septate spores are borne. The spores are hyalin, finely granular, and

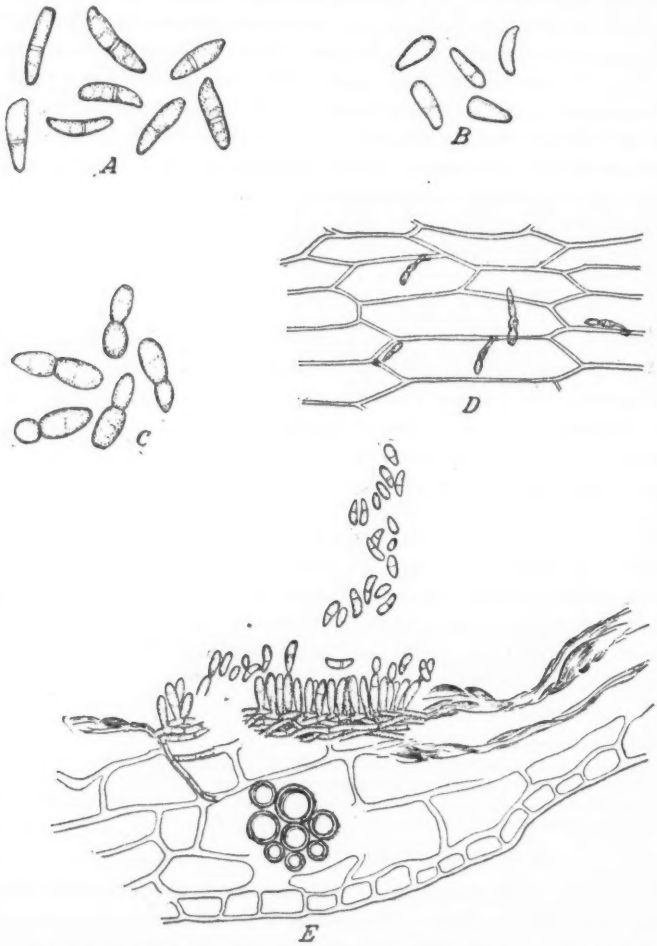

FIG. 3.—*Marssonina panattoniana: A*, Mature spores (× 750); *B*, immature spores (× 750); *C*, spores swollen and constricted at the septa just previous to germination (× 750); *D*, germinating spores on epidermal cells of lettuce leaf, showing method of penetration (× 250); *E*, cross-section of an acervulus drawn from a photomicrograph (× 300).

contain one or two large oil drops. They are constricted at the septum, and the basal cell usually shows a mark where it was attached to the conidiophore. The two cells are about equal in size. The spores average about 4 by 17 μ in size. The immature spores lack the septum and are

much smaller than mature spores. No higher form of fructification is known.

PHYSIOLOGICAL RELATIONS OF THE CAUSAL ORGANISM

GERMINATION PHENOMENA

In order to study the germination phenomena, a suspension of spores in very thin prune-juice agar was prepared, and a loopful of the preparation conveyed to each of a number of cover glasses. These were inverted over Van Tieghem rings and placed on the stage of a compound microscope. After germination had started drawings were made every hour with the aid of a camera lucida to show the progress of the development of individual spores. The smear of agar on the cover glass was purposely made thin enough to prevent the germ tube and later the mycelium from growing out of focus. Complete drawings of the organism up to 40 hours old were obtained in this way. The spores, when first produced, are single-celled, hyalin, granular, with occasional oil drops, ovate or slightly larger at one end, and measure, on the average, 7 to 8 by 2.5 to 3 μ (fig. 3, B). These small immature spores begin to swell up and a cross-wall is formed, dividing the spore into two unequal cells, or less commonly into two equal cells (fig. 3, A). The spore becomes constricted at the septum, and may be slightly curved; but many of them remain straight (fig. 3, C). When completely mature and ready to germinate, the spores measure, on the average, 4 by 17 μ. Individual spores may exceed or fall short of this size by a few microns, but they are fairly uniform in size. When spores are placed in a favorable medium (prune-juice agar was used with good results), development commences at once, the spores enlarge, and in six to eight hours put forth a short, thick germ tube, which apparently may originate at the sides or end of either cell. This quickly enlarges until it is the size of the cell producing it. When it is sufficiently elongated, a cross-wall is formed, cutting off an additional end cell. This in turn elongates, another cross-wall is formed, and thus cell by cell a long strand of mycelium is formed. It may be four or five cells long two hours after germination (fig. 1, B). Sometimes an additional germ tube may arise from the same cell (fig. 1, B, c) or from the other cell of the original 2-celled spore (fig. 1, B, f), which develops in the same manner as the first one. Branches may spring from any of the newly developed middle cells of the chain, and eight hours after germination a considerable ramification is often observed (fig. 1, E). In some cases chlamydospores are formed (fig. 1, B, d), but this phenomenon seems to depend upon the nature of the medium. Thirty hours after germination a dense network of mycelium has developed and numerous conidia are being formed. These arise, one at a time, and separately—that is, not in chains—from the apical end of short conidiophores (fig. 4). The conidiophores are usually three or four cells long, and seem identical in the shape and size of their cells with ordinary vegetative mycelium. They were not ob-

served to be club-shaped as described by Appel and Laibach (1), but this may be due to differences in the character of the medium used. A gelatinous exudate seemed to accompany the production of spores, which sometimes held numbers of spores together in a perfect sphere, probably due to surface tension, at the end of the condidiophore (fig. 3, A). After a sufficient number of spores had been produced, this tension evidently lessened, allowing the spores to scatter. The entire period from the unripe spore to the mature stage again producing spores was only 40 hours.

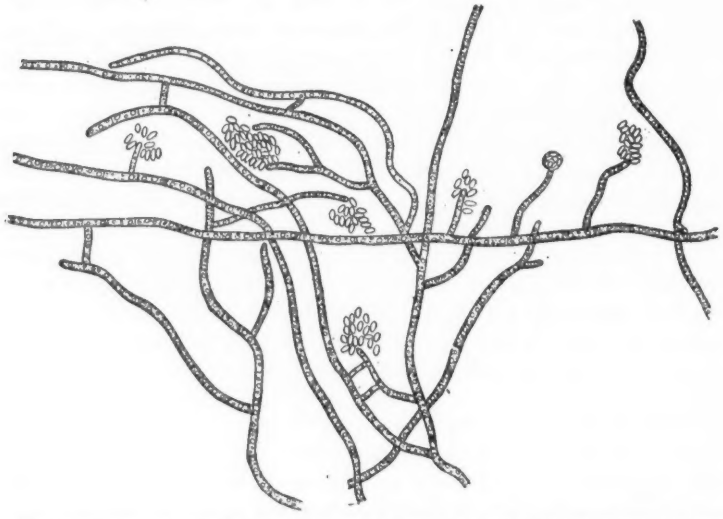

FIG. 4.—*Marssonina panattoniana:* Mycelium, condiophores, and conidia produced on prune-juice agar, 40 hours after the spores were sown (×250).

GROWTH ON VARIOUS MEDIA

Pure cultures of the fungus were transferred to a number of different kinds of media in test tubes to study its reaction to various diets. The use of so many different kinds of media seems now to have been useless labor, as very little difference was observed in the character of growth, except on widely different media. The cultures were all started on the same day, and the results recorded on the fifth day following (Table I).

TABLE I.—*Growth of Marssonina panattoniana in test tubes on various media*

Medium.	Height of growth.	Density.	Rate of growth.	Spore.	Chlamydospore.	Color.
	Mm.					
Prune agar, +30°	1.5	Thick	Rapid	Many	Produced	White.
Pear agar	.1	Sparse	do	do	Not produced	Do.
Nutrient agar	.5	Medium	Medium	Few	do	Yellowish white.
Parsnip plug	3–4	Very dense	do	Many	do	White.
Carrot plug	1	Thick	Rapid	Few	do	Do.
Potato plug	3–4	Very dense	do	Many	do	Do.
Glucose agar	2.0	do	Slow	Few	do	Do.
Prune agar, +13°	1.0	Thick	Medium	Many	do	Do.
Lettuce agar		Dense	Rapid	do	Produced	Hyalin.
Nutrient agar	.5	do	Slow	Few	Not produced	White.
Corn-meal agar	.5	do	Medium	Many	do	Do.
Corn meal	1.0	do	Rapid	Few	do	Pinkish white.
Oatmeal	1.0	do	do	do	do	White.

RELATION TO EXTERNAL CONDITIONS

RELATION TO DESICCATION

A drop of spore suspension, prepared by filtering a fresh culture through a sterile funnel containing cotton and glass wool, was placed on each of a number of sterile cover glasses and allowed to dry. The cover glasses were then stored in sterile moist chambers. They were removed one at a time at regular intervals, a drop of sterile water was placed on the dried spores, and the cover slip was inverted over a Van Tieghem ring. The spores were found to withstand desiccation for only four or five days (Table II).

TABLE II.—*Effect of desiccation on the viability of spores of Marssonina panattoniana.*

Trial.	Hour.												Day.						
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7
First	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	o	o
Second	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	o
Third	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	o	o

+=Germinated; o=did not germinate.

RELATION OF GERMINATION TO HEAT

The relation of spores to heat was determined by placing Van Tieghem cells containing hanging-drops of spore suspension in the compartments of a differential thermostat whose various compartments maintained even temperatures ranging through about 50 degrees. The thermostat consisted of a long galvanized-iron box 5 by 5 by 30 inches, divided transversely into compartments about 2 inches long. At either end was a somewhat larger compartment, one for ice and the other for water heated to 70° C. by means of a carbon-filament incandescent lamp. The box was insulated on the four sides and bottom with ground cork, and on the top with glass. The slides were hung in the center of each com-

partment on wire hammocks. Each compartment was provided with a short thermometer which could be read through the glass top. The temperatures and percentage of germination are recorded in Table III.

TABLE III.—*Relation of heat to the germination of spores of Marssonina panattoniana: Test with hanging-drop cultures in a differential thermostat*

Time.	Temperature in compartments.								
	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
First trial:	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
12 noon	60	49.5	37	34	28	22.5	17	11.5	6
5 p. m	60	45	35	32	24.5	20	15.5	11	6
6 p. m	60	44	33.5	29	24.5	20	15.5	11	6
8 p. m	60	43	33	28	24	19.5	15	11	6
Percentage of germination	0	0	0	0	66.66	50	15	10	5
Second trial:									
12 noon	58	43	37	32	27	21.5	16	11	5.5
2 p. m	58	44	37.5	31	24	20	15	10.5	5.5
8 p. m	58	44	37	30	24	20	15	10.5	5.5
Percentage of germination	0	0	0	0	85	54	13	30	5

It is readily seen that the fungus does not develop in even moderately high temperatures. This is consistent with the behavior of the fungus in the field and in other laboratory experiments.

THERMAL DEATH POINT OF SPORES

A spore suspension was prepared from a fresh culture and drawn up into capillary glass tubes which were sealed at both ends by flaming in a Meeker burner.[1] These were subjected to various constant temperatures in water baths for 10 minutes and then "shot" into test tubes of melted agar by breaking one end and applying a flame to the other end, after which plates were poured. The results are given in Table IV.

TABLE IV.—*Determination of thermal death point of spores of Marssonina panattoniana in capillary tubes*

No.	Temperature.	Number of colonies.	Remarks.	No.	Temperature.	Number of colonies.	Remarks.
	°C.				°C.		
1	35	About 200		16	42	0	
2	35	About 200		17	42	0	
3	35	About 200	Slight contamination.	18	42	0	
4	38	About 150		19	43	0	
5	38	0	Spores probably failed to "shoot" into agar.	20	43	0	
				21	43	0	
6	38	About 150		22	44	0	
7	39	61		23	44	0	Contaminated.
8	39	64		24	44	0	
9	39	17	Contaminated.	25	45	0	
10	40	0	Thermal death point.	26	45	0	
11	40	0	3 colonies of bacteria.	27	45	0	
12	40	0		28	50	0	
13	41	0		29	50	0	
14	41	0		30	50	0	
15	41	0					

[1] Novy method (LEVIN, Ezra. THE LEAF SPOT DISEASE OF TOMATO. Mich. Agr. Exp. Sta. Tech. Bul. 25, p. 21, 1916).

According to the method of determining the thermal death point by subjecting spore suspensions of the organism in thin-walled capillaries to various temperatures for 10 minutes, it is seen that the spores of this fungus have a thermal death point of 40° C. for that exposure. The experiment preceding this one showed that their germination did not take place at a much lower temperature. The failure of infection experiments when high temperatures were involved is thus readily explained.

RELATION OF OXYGEN TO GROWTH

A suspension of spores of *Marssonina panattoniana* was prepared from a freshly transferred culture by filtering the entire contents of the test tube through a sterile glass funnel containing glass wool and cotton. A microscopic examination showed that masses of mycelium were separated from the spores by this process. These were then introduced into a test tube of sterile prune-juice agar (+3, Fuller's scale), liquefied at 25° C., and thoroughly shaken. The test tube was then laid away with a slanting surface to cool. After two days the spores at the surface had germinated and made a luxuriant growth, but those in the interior of the medium had ceased to grow immediately after germination. It may be concluded from this that considerable free oxygen is necessary for the development of the fungus.

RECOMMENDATIONS FOR CONTROL

The experiments reported in this paper have shown the usual source of infection of the anthracnose of lettuce, the type of dissemination of the organism, and the relation of the fungus to certain environmental factors. A rational system of control can be based upon these findings.

The anthracnose in the greenhouse or the field commonly starts from the trash of a preceding diseased crop. In the field, rotation is possible, and this, if coupled with the avoidance of manure containing lettuce refuse, will eliminate two important sources of the disease. If further work should show that the fungus is carried with the seed, then some simple system of seed disinfection can probably be readily applied. In the greenhouse, rotation is for the most part impractical. Here careful sanitary measures must prevail. The common practice in greenhouses is to turn under the refuse from a previous crop and to plant immediately in the soil. Naturally with this type of culture there is abundant chance for the infection of the growing crop. Careful collection and destruction of lettuce refuse is recommended. The fertilizer value of the leaves turned under is negligible.

The grower should inspect his beds throughout the growing season, and any plant showing anthracnose, drop, or other severe diseases should be promptly removed from the bed. One plant with the anthracnose can become a center of infection which may involve a whole bed.

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Such diseased plants should not be thrown on the manure or compost heap. No doubt the best method of disposing of them is to burn them.

It can not be said that this collection of diseased material can be relied upon to do more than remove the gross sources of infection. It is, furthermore, a preventive measure which is extremely effective against the other lettuce diseases, notably, lettuce-drop and lettuce-graymold.

This disease is commonly found where the splashing or the dripping of water occurs. The repair of gutters or of valves is of obvious necessity. Few growers realize fully the extent of damage which comes from this source. It is not uncommon to see a 10 per cent loss due to the dripping of water the full length of a house. The sprinkling of plants with the hose is an inefficient way of watering. It is rarely necessary to rinse off the leaves of plants in beds, and when hose watering is necessary, the water can be safely and quickly applied by flowing the stream along the ground. Where soil conditions permit, subirrigation is an excellent method.

The most popular method of greenhouse watering is by some type of overhead system. It might seem at first glance that this method would have the same disadvantage as sprinkling with a hose. It has been found by observation that in the overhead systems the splashing from plant to plant, or even from leaf to leaf, does not occur. The jets of water are too fine and the force too slight when the water reaches the plant to bring about any splashing. The writer has no knowledge of an outbreak of lettuce anthracnose in greenhouses watered by the overhead system. The only diseased plants found with such watering systems have always been associated with leaky valves or leaky gutters. The glutinous nature of the spores of the anthracnose fungus explains why these bodies can not be transferred from plants except when the diseased spot is soaked with water. In the field, cultivation should be avoided when the plants are wet. The transference of the disease in the greenhouse is almost wholly brought about by spreading of water. The experiment reported and the diagrams (fig. 2) show most conclusively the relation of this factor.

The recommendation, therefore, is to do away with splashing of water from plant to plant or leaf to leaf. The grower may accomplish this in several ways. The overhead systems are efficient, and are undoubtedly the most popular method of greenhouse watering.

There is possible also the adjustment of the growing conditions so as to favor the lettuce plant and to check the fungus. The most efficient agent in this regard is ventilation. The spores of the fungus will probably not germinate and enter into the lettuce leaf when transferred, unless the leaves stay wet for a considerable time—6 to 12 hours. Since ventilation can not wholly compensate for heavy watering, and as humid conditions will permit germination and penetration of the leaves, excessive watering should be avoided. The most successful lettuce growers aim to keep the

plants, as they say, "on the dry side." This measure is of general benefit with other diseases as well.

High temperature will also check the anthracnose. Lettuce, however, will not make its best growth under such conditions. Hence, the utilization of this factor to prevent the occurrence of the disease is probably of but slight benefit. No doubt in cases of epidemics the disease could be effectively checked by raising the temperature to 30° C. (80° or 90° F.), especially if this were coupled with good ventilation.

So far, the measures outlined have been preventive. These are cheap and readily applied and in line with the best commercial practice. There is left a direct method of fighting this disease. It is possible to protect leaves of lettuce from this disease by spraying them with a protective coat such as Bordeaux mixture or ammoniacal copper carbonate. The latter, while not so effective as fungicide, avoids the staining which accompanies the use of Bordeaux mixture. This method is to be used as a last resort and should not be necessary if the other preventive measures are followed.

SUMMARY

This paper gives the results of experiments with the anthracnose of lettuce and its causal organism.

The signs of the disease consist, in general, of distinct lesions on the leaf blade and midrib. This is frequently accompanied by dwarfing and wilting. Previous workers had found *Marssonina panattoniana* (*M. perforans*) associated with this disease and assumed its etiological relation. This relation has been proved.

A study of the conditions of infection showed that germ tubes were produced from spores in the average time of six hours. Penetration takes place at a point of contact of the walls of the epidermis. Temperatures above 32° C. prevent germination and infection, but excessively wet conditions are not necessary for infection.

Germination phenomena and the relation to various nutrient media were determined. The spores withstand desiccation on glass only four or five days. The fungus does not grow at temperatures above 30° C. The thermal death point was found to be 40° for 10 minutes. The organism does not grow submerged in agar.

The source of the organism in new locations is as yet unsolved. The trash from a previously diseased crop is undoubtedly the chief agent in carrying the disease over from year to year. Splashing, as from a hose in watering, proved to be a ready means of spreading the disease.

Control measures, chiefly prophylactic, based on the findings of this paper are given.

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PLATE C

Leaf of lettuce with lesions of *Marssonina panattoniana*.

PLATE 20

Marssonina panattoniana:

A.—Lettuce leaves of Grand Rapids Forcing variety, showing lesions on midrib and blade.

B.—Lesions on midrib; slightly enlarged.

C.—Lesions on leaves of Black-Seeded Tennis Ball variety produced by artificial inoculation.

A
B
C

THE CALCIUM ARSENATES

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INTRODUCTION

Investigations during the past years on the preparation of the calcium arsenates, together with a study of their chemical and physical properties, indicate the possibility of an economic substitute for the arsenates of lead as an insecticide. During the past two decades the value of the lead arsenates as a stomachic insecticide has been demonstrated. Recently, owing perhaps to conditions brought on by the world war, the cost of this necessary spray material has advanced in price to such an extent that there is a possibility of the curtailing of its use, to the detriment of our orchards and the quality of crops produced.

Heretofore numerous field experiments have been made throughout various sections of the United States to ascertain the practicability of using calcium arsenate as a spray. In most cases these trial experiments have failed, owing to excessive burning of the foliage. As with most arsenicals, the cause of the burn is due to the action of arsenic upon the foliage. The high water-soluble content of commercial samples of calcium arsenate indicates the possibility that therein lies the cause of the intensive burning of foliage. This difficulty was encountered when the commercial lead arsenates were first used, but a study of the properties of laboratory-prepared salts enabled manufacturers to produce a high-grade insoluble lead arsenate that gave orchardists no trouble and caused very little burning. Consequently, it was thought that a more complete knowledge of the composition of the calcium arsenates and a study of the methods of preparation and physical and chemical properties would give an insight relative to the practicability of their use as a substitute for the arsenates of lead.

EXPERIMENTAL WORK

A review of the literature relative to compounds of calcium and arsenic shows that numerous preparations are discussed from a theoretical standpoint, but few have actually been obtained in the laboratory. The calcium arsenates that are of vital interest to us from an insecticidal standpoint must necessarily have such physical and chemical properties that indicate a fairly stable salt. Preliminary experiments convinced us that two salts, the tricalcium arsenate [$Ca_3(AsO_4)_2$] and the calcium hydrogen arsenate ($CaHAsO_4$) appeared to be the only favorable ones.

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Recent work on these two salts is very limited. In 1844 Rammelsberg [1] prepared the pure salt corresponding to the theoretical composition $CaHAsO_4.2H_2O$, containing two molecules of water of crystallization which was further substantiated at about the same date by the work of Klaproth [1] and Dufet.[1] Others, at even an earlier date, obtained different results, indicating variation in amounts of water of crystallization and constitution. Although citations are made to possible methods of preparing the tricalcium arsenate, no data containing actual figures of analysis were found. Furthermore, numerous analyses of commercial samples, both the so-called chemically pure salts, intended for reagents in chemical work, and those sold by manufacturers for spraying purposes proved to be mixtures of various calcium salts, and no two were alike in composition. This point will be further commented upon later.

Previous investigators suggest the preparation of calcium hydrogen arsenate by using calcium chlorid ($CaCl_2$) and sodium hydrogen arsenate (Na_2HAsO_4), the reaction being in accordance with the following equation:

$$CaCl_2 + Na_2HAsO_4 \rightarrow CaHAsO_4 + 2NaCl$$

These salts may, however, also react as represented in the following equation, giving the tricalcium arsenate:

$$3CaCl_2 + 2Na_2HAsO_4 \rightarrow Ca_3(AsO_4)_2 + 4NaCl + 2HCl$$

It is obvious, therefore, that both the arsenates may be formed in greater or less amounts when prepared in the above manner. This may account for the variable composition of the commercial arsenates noted above, and emphasizes more strongly the necessity of specific control of conditions in such a manner that one or the other salt will be produced.

PREPARATION OF CALCIUM HYDROGEN ARSENATE AND TRICALCIUM ARSENATE

In order to make a complete study of those physical and chemical properties of the calcium arsenates that would have an immediate bearing upon their value as a spray material, the preparation of these salts in very pure form is essential.

Numerous methods of preparation were undertaken, but in most cases mixtures were obtained of varying composition. After trial experiments the following method was found to be satisfactory:

Solutions of calcium chlorid and sodium hydrogen arsenate were made, and very slightly acidified with either acetic, hydrochloric, or nitric acid. After filtration, to obtain absolutely clear solutions, the cold calcium-chlorid solution was gradually poured into the sodium-

[1] GMELIN, Leopold, and KRAUT, K. J. HANDBUCH DER ANORGANISCHEN CHEMIE. Aufl. 7, Bd. 3, Abt. 2, p. 569. Heidelberg, 1908. Cites Rammelsberg, Klaproth, and Dufet.

hydrogen-arsenate solution, the solution being constantly stirred. A heavy voluminous precipitate formed immediately and slowly settled; the clear supernatant liquid was decanted off. It was further washed by decantation several times and finally was brought upon a Buchner filter and washed with hot distilled water until free of chlorids. The pure white amorphous powder was then dried at 100° C.

The combined washings were evaporated down to a small volume, when crystals of calcium hydrogen arsenate separated out. After removing the mother liquor by suction, the crystals were washed several times with small quantities of boiling water and dried at 100°.

Analyses made of the samples thus obtained are as follows:

	Total percentage.
Powder:	
Calcium as CaO	28.14
Arsenic as arsenic pentoxid	57.91
Crystals:	
Calcium as CaO	28.40
Arsenic as As_2O_5	58.15
Theoretical composition of $CaHAsO_4.H_2O$:	
Calcium as CaO	28.24
Arsenic as As_2O_5	58.11

Comparing the results with the theoretical composition of $CaHAsO_4.H_2O$ as noted above, we conclude that both samples are pure calcium hydrogen arsenate, containing one molecule of water of crystallization.

When these samples were further dried at 175° C., both lost their water of crystallization, as shown by the following analytical results:

	Total percentage.
Powder:	
Calcium as CaO	31.10
Arsenic as As_2O_5	63.80
Crystals:	
Calcium as CaO	31.20
Arsenic as As_2O_5	63.83
Theoretical composition of $CaHAsO_4$:	
Calcium as CaO	31.13
Arsenic as As_2O_5	63.86

A comparison of these results with the theoretical composition of anhydrous calcium hydrogen arsenate shows each of the samples to be a pure calcium hydrogen arsenate.

When the heating is continued at 230°, the tendency to form the pyroarsenate ($Ca_2As_2O_7$) is observed as shown by the analysis:

	Total percentage.
Calcium as CaO	32.01
Arsenic as As_2O_5	65.78

Another method found successful in preparing pure calcium hydrogen arsenate consists in dissolving a commercial calcium arsenate, which may be a mixture of the two calcium arsenates under discussion, in the smallest quantity possible of either hydrochloric or acetic acid, as large a volume of water as convenient being maintained. When evaporated upon a hot plate, either amorphous powder or crystals separate out, depending upon rapidity of evaporation.

A simple laboratory method that also proved successful consists in adding slowly a solution of calcium hydroxid [$Ca(OH)_2$] to a clear aqueous solution of arsenic acid, and in stirring vigorously meanwhile, until the acid is about three-fourths neutralized. By slow evaporation crystals of pure calcium hydrogen arsenate separate out.

The chief precaution that must be observed in order to obtain the pure salt is to maintain an excess of acid or H-ion in the original solutions. This excess of acid, however, must be at a minimum, or the precipitate will redissolve.

In the preparation of tricalcium arsenate three methods suggest themselves from a theoretical standpoint, as indicated by the following equations:

(1) When pure calcium hydrogen arsenate is acted upon by an alkali:

$$3CaHAsO_4 + 2NaOH \rightarrow Ca_3(AsO_4)_2 + Na_2HAsO_4 + 2H_2O$$

(2) When arsenic acid is completely neutralized with a solution of calcium hydroxid:

$$2H_3AsO_4 + 3Ca(OH)_2 \rightarrow Ca_3(AsO_4)_2 + 6H_2O$$

(3) When solutions of calcium chlorid and sodium arsenate are allowed to react:

$$3CaCl_2 + 2Na_3AsO_4 \rightarrow Ca_3(AsO_4)_2 + 6NaCl$$

In all cases cited, if the reaction is permitted to reach an equilibrium and conditions are controlled as indicated, it is possible pure tricalcium arsenate would result. However, the last proved to be the most practical, and concordant results were obtained. The perfected method is as follows: Cold solutions of both calcium chlorid and sodium hydrogen arsenate were prepared and the former made just sufficiently alkaline that no calcium hydroxid precipitated out. To the sodium-hydrogen-arsenate solution just enough sodium hydroxid was added to change completely the salt to sodium arsenate. (Special care must be taken lest too much sodium hydroxid should be added, as this would form calcium hydroxid when combined with the calcium-chlorid solution.) Both solutions were then filtered and the clear calcium-chlorid solution was added to the sodium-arsenate solution. A heavy voluminous precipitate formed that settled very slowly. This was washed by repeated centrifuging and decanting off the supernatant liquid each

time. It was finally brought upon a Buchner filter and washed free of chlorids with hot water. After drying in an oven at 100°, the analysis gave the following results:

	Total percentage.
Calcium as CaO	38.49
Arsenic as As_2O_5	52.50

These results indicate that a salt is produced containing two molecules of water of crystallization and agreeing with the formula $Ca_3(AsO_4)_2 \cdot 2H_2O$

When further dried at 175°, the pure calcium arsenate was obtained as shown by the following analytical data:

	Total percentage.
By analysis:	
Calcium as CaO	42.16
Arsenic as As_2O_5	57.73
Theoretical composition of $Ca_3(AsO_4)_2$:	
Calcium as CaO	42.20
Arsenic as As_2O_5	57.80

SPECIFIC GRAVITY, SOLUBILITY, AND RELATIVE STABILITY OF CALCIUM HYDROGEN ARSENATE AND TRICALCIUM ARSENATE

A spray material in powder form must be in a fine state of subdivision to facilitate efficient spreading on foliage and must have a specific gravity sufficiently low that it will remain in suspension in water for a considerable length of time.

The specific gravity was obtained by determining the weight of absolute alcohol displaced by a known quantity of the salt. The alcohol was specially prepared by redistilling the commercial chemically pure absolute alcohol over calcium oxid obtained by igniting chemically pure calcium carbonate ($CaCO_3$). All weighings were made at 20° C. with recently standardized weights. The specific gravity for the different salts was found to be as follows:

Calcium hydrogen arsenate ($CaHAsO_4 \cdot H_2O$) at 20°/4°	3.09
Anhydrous calcium hydrogen arsenate ($CaHAsO_4$) at 20°/4°	3.48
Tricalcium arsenate [$Ca_3(AsO_4)_2 \cdot H_2O$] at 20°/4°	3.23
Anhydrous tricalcium arsenate [$Ca_3(AsO_4)_2$] at 20°/4°	3.31

The solubility of an arsenical utilized for insecticidal purposes is an exceedingly important factor. The presence of an excess of soluble arsenic or any salt that would ultimately pass into solution, forming arsenic acid or a soluble arsenate, would cause burning of foliage and render the arsenical useless for spraying purposes. Qualitative tests showed that both calcium hydrogen arsenate and tricalcium arsenate were slightly soluble, and, like many other calcium salts, the solubility is greater at lower than at higher temperatures. The determinations were made at 25° C. in a constant-temperature bath that was fitted with a revolving bottle holder. By means of the latter, four bottles could be submerged and kept in a state of constant agitation during the

entire experiment. Various amounts of the samples were introduced into the different bottles, and 300 c. c. of conductivity water were added to each. They were then placed in the constant-temperature bath and allowed to revolve for five days, when 100 c. c. were removed from each bottle and evaporated on a steam bath. The bottles were replaced in the bath and allowed to continue three days longer, when another 100 c. c. was removed and evaporated as before.

Finally, after evaporation the salts were dried in an electric oven at 100° C. and then at 175°, as recorded in Table I.

TABLE I.—*Comparative solubility at 25 °C. of calcium hydrogen arsenate and tricalcium arsenate*

Salt.	Drying temperature.	Weight (grams in 100 gms. of water).
	°C.	
Calcium hydrogen arsenate	100	0.3308
Do	175	.3108
Tricalcium arsenate	100	.0140
Do	175	.0133

Analysis of the dissolved material showed that the composition of the latter was the same as that of the original salt used, indicating no perceptible hydrolysis.

We observe from the results given in Table I that at 25° C. the calcium hydrogen arsenate is far more soluble than the tricalcium arsenate. This point is of considerable practical importance, since the solubility of the calcium hydrogen arsenate proximates the amount shown by field experiments to cause burning of foliage. These data indicate the futility of attempting to use the pure calcium hydrogen arsenate alone as a spray. The tricalcium arsenate, on the other hand, is only slightly soluble, and the danger of burning from that source would probably be negligible.

From the standpoint of density and solubility, it is probable that the tricalcium arsenate could be safely used as a spray material, while the calcium hydrogen arsenate is doubtful. An important factor remains yet to be taken into consideration, namely, the stability of the salts. This was ascertained by a study of the chemical change that resulted when either the calcium hydrogen arsenate or tricalcium arsenate was shaken at intervals for several days in solutions of acids, both organic and inorganic, ammonium hydroxid, sodium hydroxid, sodium chlorid, and similar salts. In all cases tried the calcium hydrogen arsenate reacted, dissolved, or arsenic in soluble form was found in solution. However, the tricalcium arsenate manifested greater stability and in many instances showed only slight reactivity. Inferring from the relative stability of the two salts, both arsenates would under severe and abnormal climatic conditions probably yield to the action of carbon dioxid and moisture in

the air with the consequent formation of free arsenic acid. The tricalcium arsenate being more insoluble and more stable than the calcium hydrogen arsenate would not have the tendency to react so readily and the danger attending its use would be diminished accordingly.

EFFECT OF CALCIUM HYDROXID ON SOLUBILITY OF THE CALCIUM ARSENATES

Our investigations thus far indicate favorable possibilities for the use of the calcium arsenates. The presence of any substance that would prevent solubility and reactivity would prove beneficial in the use of tricalcium arsenate and almost a necessity in the case of the calcium hydrogen arsenate. To judge from a theoretical standpoint, ordinary quicklime (CaO) would fulfill these requirements. The calcium hydroxid, on becoming soluble, would react with any arsenic that goes into solution, forming more calcium arsenate.

In order to corroborate the above assumption, different amounts of the pure salts were introduced into each of several 200-c. c. graduated flasks together with known quantities of lime (CaO). In some cases calcium carbonate ($CaCO_3$) was also added. The flasks were then made up to mark with distilled water and shaken at intervals for two days. After allowing the salts to settle, determinations were made for total arsenic and calcium in the clear supernatant liquid. The results are given in Table II.

TABLE II.—*Effect of calcium hydroxid on the solubility of the calcium arsenates. Time, two days*

Flask No.	Substances used.	Quantity added.	Quantity of arsenic pentoxid found.	Quantity of calcium oxid found.
		Gm.	*Gm.*	*Gm.*
I	Tricalcium arsenate	1.0	0.0084	0.0049
	Calcium oxid	.2		
	Calcium carbonate	.8		
II	Tricalcium arsenate	1.5	None.	.1131
	Calcium oxid	.7		
	Calcium carbonate	None.		
III	Calcium hydrogen arsenate	1.0	None.	.0554
	Calcium oxid	.5		
	Calcium carbonate	None.		
IV	Calcium hydrogen arsenate	1.0	.0026	.0082
	Calcium oxid	.2		
	Calcium carbonate	.8		
V	Calcium arsenate, C. P. (J. T. Baker)	2.0	None.	.0573
	Calcium oxid	1.0		

Inspection of the results given in Table II indicates that wherever calcium oxid is present in even slight excess, so that calcium hydroxid was found qualitatively in solution, no soluble arsenic was detected.

Furthermore, the calcium carbonate prevented in no way the solubility of the calcium arsenates. It is quite evident, therefore, that if there is an excess of calcium hydroxid in the system, the solubility of both calcium arsenates is inhibited.

In order to substantiate the former results and to ascertain whether or not, by allowing the action to continue for a longer time, the system would come to an equilibrium and continue indefinitely with no arsenic in the solution, a similar set of flasks was prepared. These were allowed to stand for two weeks, with an occasional shaking, and then the supernatent liquid was analyzed as before. The quantity of material used, together with the results obtained, is given in Table III.

TABLE III.—*Effect of calcium hydroxid on the solubility of the calcium arsenates. Time, two weeks*

Flask No.	Substances used.	Quantity added.	Quantity of arsenic pentoxid found.	Quantity of calcium oxid found.
		Gm.	*Gm.*	*Gm.*
I	Tricalcium arsenate	1.0	None.	0.2481
	Calcium oxid	.5		
II	Calcium hydrogen arsenate	1.0	None.	.2451
	Calcium oxid	.5		
III	Calcium hydrogen arsenate	1.0	None.	.2540
	Calcium oxid	1.0		

The results given in Table III further verifies those in the previous experiment and emphasizes the fact that a definite point of equilibrium is reached in which the concentration of calcium hydroxid in solution becomes constant. Furthermore, it was found that the concentration of calcium hydroxid is the same as the maximum solubility of calcium hydroxid in pure water at the same temperature.

From a practical standpoint the preventive action of calcium hydroxid on the solubility of the calcium arsenates is of vital importance. The free calcium hydroxid has no harmful effects upon foliage. If, therefore, 1 part of quicklime is added for every 2 parts of either calcium hydrogen arsenate or tricalcium arsenate as a combination spray, no burning of foliage should result from the solubility of the arsenates. Furthermore, the action under severe atmospheric conditions will be greatly diminished, since the carbon dioxid in the air will first react with the calcium hydroxid, forming the harmless calcium carbonate before the calcium arsenates are effected.

ACTION OF CARBONIC ACID UPON THE CALCIUM ARSENATES

A study of the action of carbon dioxid upon the calcium arsenates is an important consideration. Although chemical changes due to carbonic acid, or a saturated water solution of carbon dioxid, as noted in

laboratory experiments, would be more vigorous than the action of the carbon dioxid of the atmosphere, a similar but much slower change would probably occur. In order to ascertain whether any reaction may obtain, a series of flasks, each of which contained a specific quantity of calcium arsenate and 50 c. c. of distilled water was prepared. Carbon dioxid was then passed through the mixture, keeping the salts in a constant state of agitation. After 10 hours the supernatant liquid was boiled to expel excess carbon dioxid and a determination was made for arsenic in solution. The results, together with amounts of arsenate used, are given in Table IV.

TABLE IV.—*Effect of carbolic acid on the solubility of the arsenates*

Salt used.	Quantity taken.	Quantity of arsenic as arsenic pentoxid in 50 c. c.
	Gm.	*Gm.*
Tricalcium arsenate	2	0.0208
Calcium hydrogen arsenate	2	.0428
Tricalcium arsenate+0.5 gm. of calcium oxid	1	.0012
Calcium hydrogen arsenate+0.5 gm. of calcium oxid	1	.0046
Calcium hydrogen arsenate+1 gm. of calcium oxid	1	.0020

From the above results it is plainly evident that carbonic acid has a solvent action upon the calcium arsenate. Furthermore, when the excess carbon dioxid was boiled off, a precipitate, consisting of a mixture of calcium carbonate and a smaller amount of calcium arsenate, was thrown down. The presence of calcium hydroxid, however, diminished the solvent action appreciably.

REACTION BETWEEN THE CALCIUM ARSENATES AND LIME-SULPHUR SOLUTION

Combination sprays are of great economic importance, since time, labor, and money are thereby saved. The tendency for chemical reaction that destroys or greatly diminishes the efficiency of both spray materials often results, and consequently prevents their use in this manner. The combination of lead hydrogen arsenate and lime-sulphur exemplifies this difficulty, as shown by Robinson and Tartar.[1] The concentration of the lime-sulphur is reduced; arsenic is found in solution; lead sulphid is precipitated out, thus utilizing part of the lead arsenate; and severe burning of foliage may occur, depending upon weather conditions.

When pure tricalcium arsenate was added to dilute lime-sulphur, no reaction appeared to occur. Sanders[2] in field experiments reports

[1] ROBINSON, R. H., and TARTAR, H. V. THE ARSENATES OF LEAD. Oreg. Agr. Exp. Sta. Bul. 128, 32 p., 2 fig. 1915.

[2] SANDERS, G. E. THE EFFECT OF CERTAIN COMBINATIONS OF SPRAYING MATERIALS ON THE SET OF APPLES. *In* Proc. Ent. Soc. Nova Scotia, 1916, p. 17–21. 1917.

more favorable results obtained with the calcium-arsenate-lime-sulphur combination spray than with lead-arsenate-lime-sulphur. Hence, a study of the chemical reaction, if any, was initiated. Pure lime-sulphur was prepared in the laboratory from crystallized sulphur and calcium oxid, the latter being obtained by igniting calcium carbonate. The lime-sulphur produced had a specific gravity of 1.242, or 28.2° Baumé, and was diluted to average field spraying strength. Both of the calcium arsenates were prepared in the laboratory in pure form. Into each of several flasks 1 gm. of tricalcium arsenate, 1 gm. of calcium hydrogen arsenate, or 1 gm. of calcium hydrogen arsenate + 0.5 gm. of calcium oxid was introduced, and 200 c. c. of the diluted lime-sulphur were added. A control flask containing 200 c. c. of the diluted lime-sulphur only was also prepared. The flasks were then shaken at intervals during two days, after which the salts were allowed to settle and determinations were made for total sulphur, calcium oxid, and arsenic pentoxid in the supernatant lime-sulphur solution. Table V records the results, expressed in grams contained in 200 c. c.

TABLE V.—*Reaction between lime-sulphur and the calcium arsenates*

Constituent.	Control.	Lime-sulphur and tricalcium arsenate.	Lime-sulphur and calcium hydrogen arsenate.	Lime-sulphur and calcium hydrogen arsenate + calcium oxid.
	Gm.	*Gm.*	*Gm.*	*Gm.*
Sulphur ..... total..	1.8400	1.8450	1.8490	1.8420
Calcium oxid ..... total..	.8810	.8840	.8820	1.1090
Arsenic pentoxid ..... total..	.....	None.	None.	None.

The above results proved favorable beyond expectation. There appears to be no chemical change whatsoever in the lime-sulphur solution, all constituents remaining constant and agreeing with results given for the control solution. Barring the possibility of chemical reaction after spraying, we have in the calcium arsenate an ideal spray material to be used in combination with lime-sulphur. The efficiency of the latter is not reduced and the calcium arsenate remains unchanged.

Attention is further brought to the analysis of the liquid in the flask containing calcium hydrogen arsenate + calcium oxid. As with calcium hydrogen arsenate and tricalcium arsenate, no arsenic was found in the lime-sulphur solution, and the sulphur content remained constant. The amount of calcium oxid, however, exceeds that obtained in the other flasks. This increase of the calcium oxid is equivalent to the solubility of calcium hydroxid in pure water at the temperature under observation. Since the addition of lime in no way reacts with either of the other spray

materials, it might be advisable to have it present in order to prevent danger of a foliage burn due to severe atmospheric conditions. This would be especially true of the less stable calcium hydrogen arsenate. This precaution, however, must be verified by field experiments.

Since the above conclusions were obtained on pure samples, the experiment was repeated with commercial products in order to ascertain the practicability of their use. A solution of commercial lime-sulphur having a specific gravity of 1.227, or 26.7° Baumé, was diluted in the same proportion as indicated in the previous experiment. A commercial calcium arsenate was used that had the following composition:

Calcium oxid	total..	39.20 per cent.
Arsenic pentoxid	total..	48.90 per cent.
Calcium carbonate	total..	1.14 per cent.

From this analysis we infer that the sample consisted of over 60 per cent of tricalcium arsenate and the remainder calcium hydrogen arsenate and calcium carbonate.

Treatment was similar to that of the last experiment, and the composition of the lime-sulphur solution is given in Table VI.

TABLE VI.—*Reaction between commercial calcium arsenate and lime-sulphur*

Substance used.	Calcium oxid.	Sulphur.	Arsenic pentoxid.
	Gm.	*Gm.*	*Gm.*
Control (lime-sulphur only)	0.7821	1.6482	.....
Calcium arsenate and lime-sulphur	.7816	1.6474	None.

Here again, we observe that no apparent chemical action has occurred. No arsenic was found in solution, and the total calcium oxid and sulphur content remained the same. It is obvious that a commercial sample composed of both calcium hydrogen arsenate and tricalcium arsenate and possibly other impurities may be combined with commercial lime-sulphur with the same degree of safety as the pure laboratory products.

The use of dry substitutes for lime-sulphur is rapidly becoming prevalent; hence, a study of their combination with calcium arsenate would be beneficial. Consequently 0.5 gm. of a commercial "dry lime-sulphur" and the same quantity of a so-called "soluble sulphur" which is composed chiefly of the sulphids of sodium or potassium were used. Each was introduced into 200-c. c. graduated flasks, together with 1 gm. of commercial calcium arsenate, and each mixture was then made up to mark with water. After shaking occasionally during two days the mixture in both flasks was allowed to settle and was analyzed for arsenic in solution, as reported in Table VII.

TABLE VII.—*Solubility of commercial calcium arsenate used with dry lime-sulphur*

Substance used.	Arsenic pentoxid in 200 c.c.
	Gm.
Control	None.
Dry lime-sulphur	None.
Soluble sulphur	0.230

These results indicate that no chemical changes occurred when calcium arsenate and dry lime-sulphur were combined. The soluble-sulphur compound, however, caused such a large quantity of arsenic to pass into solution that would make it inadequate for utilization as a combination spray.

VALUATION OF COMMERCIAL SAMPLES

There are at present on the market various calcium-arsenate products offered as substitutes for lead arsenate, together with the so-called chemically pure salts used as reagents for chemical work. Complete analyses have been made of these samples, both to ascertain whether or not they approximated the theoretical composition of the pure salt and also to estimate their commercial value as spray materials. Table VIII gives a compilation of the results obtained.

TABLE VIII.—*Composition of samples of commercial calcium arsenate*

Sample.	Calcium oxid.	Arsenic pentoxid.	Calcium carbonate.
	Per cent.	*Per cent.*	*Per cent.*
Kahlbaum (C. P.)	36.62	51.50	Trace.
Baker (C. P.)	40.96	42.75	7.25
Commercial No. 1	43.46	40.80	40.00
Commercial No. 2	39.20	48.86	1.14
Commercial No. 3	45.61	19.22	8.40

The wide variation in the composition of commercial arsenates is plainly evident from the results given in Table VIII. Both Kahlbaum's and J. T. Baker's chemically pure samples, supposed to be high-grade salts, are mixtures of the calcium hydrogen arsenate and tricalcium arsenate, with between 10 per cent and 20 per cent water of crystallization or constitution. Furthermore, both the Kahlbaum and Baker samples contained some calcium carbonate. Commercial samples 1, 2, and 3 likewise emphasize the necessity of greater care in their manufacture. Attention is especially called to sample 1. It contains 40 per cent of calcium carbonate, but only 43.46 per cent of total calcium, estimated as calcium oxid. These figures indicate that there is insuffi-

cient base to combine with the quantity of arsenic pentoxid present, and further examination showed the latter was there as actual arsenic pentoxid uncombined. It is probable that the manufacturer attempted to prepare the calcium arsenate from arsenic pentoxid and an impure lime which was chiefly calcium carbonate. The latter would react very slowly, if at all, and the excess arsenic pentoxid over the amount required to combine with the calcium hydroxid would remain unchanged as noted above. If the manufacturer would exercise a more complete control of conditions and know definitely what materials are being used, a spray material sufficiently pure for practical use could probably be obtained.

Thus far our investigation has shown the possibility of the calcium arsenates' being a practical, economical, and evidently satisfactory substitute for the more expensive lead arsenate. The pure salts contain 57.8 and 63.9 per cent of arsenic calculated as arsenic pentoxid for the tricalcium arsenate and calcium hydrogen arsenate, respectively, while the hydrogen and basic lead arsenate contains only 33.1 and 25.5 per cent of arsenic pentoxid, respectively; in other words, the calcium arsenates contain more than twice as much arsenic, the active killing agent of the insecticide. For practical spraying purposes, therefore, only one-half of the quantity of calcium arsenate should be used, compared with lead arsenate. This point must, however, be more definitely determined by field experiments, since there is a bare possibility that the lead has toxic values that the calcium does not have. Relative toxic values and killing efficiency has been recently shown in laboratory experiments with the "common tent caterpillar" by Lovett and Robinson [1] and with the "fall webworm" by Scott and Siegler,[2] indicating equal killing efficiency of calcium arsenate compared with lead arsenate.

Field spraying experiments have been carried on by several investigators with more or less successful results. This may be due to the use of an unreliable calcium arsenate similar to the one cited above. Sanders,[3] however, reports very favorable results during the past two seasons. Likewise, Scott and Siegler [4] obtained encouraging results.[5]

In cooperation with Prof. A. L. Lovett, Entomologist at this Station, preliminary field experiments were tried with the pure salts prepared in the laboratory. Favorable results, especially with tricalcium arsenate plus calcium oxid were obtained, but owing to insufficient time and late-

[1] LOVETT, A. L., and ROBINSON, R. H. TOXIC VALUES AND KILLING EFFICIENCY OF THE ARSENATES. *In* Jour. Agr. Research, v. 10, no. 4, p. 199-207. 1917.

[2] SCOTT, E. W., and SIEGLER, E. H. MISCELLANEOUS INSECTICIDE INVESTIGATIONS. U. S. Dept. Agr. Bul. 278, 47 p. 1915.

[3] SANDERS, G. E. ARSENATE OF LEAD VS. ARSENATE OF LIME. *In* Proc. Ent. Soc. Nova Scotia, 1916, p. 40-45. 1917.

[4] SCOTT, E. W., and SIEGLER, E. H. Op. cit.

[5] Scott and Siegler gave the analysis of the calcium arsenate used as showing only 0.04 per cent of soluble arsenic oxid. A possible explanation is that the spray material was prepared from stone lime (containing 80 per cent of calcium oxid), sodium arsenate, and water. A very slight excess of the stone lime would prevent any arsenic from becoming soluble, as shown in our experiments reported above.

ness in the season, the trials were limited. During the coming spraying season more elaborate field experiments will be conducted by Prof. Lovett and reported upon at a later date.

SUMMARY

(1) This paper reports a chemical study of the calcium arsenates.

(2) Pure calcium hydrogen arsenate ($CaHAsO_4$) and tricalcium arsenate [$Ca_3(AsO_4)_2$] have been prepared and methods for their preparation outlined.

(3) The specific gravity for calcium hydrogen arsenate was found to be 3.48; that for tricalcium arsenate 3.31.

(4) The solubility of calcium hydrogen arsenate in 100 gm. of water at 25° was 0.310 gm. and that of tricalcium arsenate was 0.013 gm.

(5) A chemical study of the relative stability showed that (a) there was no apparent reaction between either calcium hydrogen arsenate or tricalcium arsenate and lime-sulphur when combined at a dilution used in field spraying. (b) The addition of excess of calcium oxid to either of the calcium arsenates prevented arsenic from going into solution. (c) Some commercial substitutes for lime-sulphur reacted with both of the calcium arsenates. (d) The arsenates reacted with or became soluble in organic acids and various salts, such as sodium chlorid.

(6) The composition of various commercial arsenates is given and commented upon.

STEMPHYLIUM LEAFSPOT OF CUCUMBERS[1]

By GEORGE A. OSNER[2]

Associate Botanist, Purdue University Agricultural Experiment Station, and Collaborator, Cotton and Truck Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the latter part of September, 1915, the writer discovered a peculiar leafspot on cucumbers (*Cucumis sativus*) in a small garden near Plymouth, Indiana. The spots showed a distinctly mottled effect, and in some cases were so numerous as to entirely kill the leaf. Specimens of this same disease were discovered about the same time by Mr. W. W. Gilbert, in two cucumber fields, one at Lapaz and the other at Lakeville, Ind., and later, in a field at Bowling Green, Ohio. A field was found a few miles from Plymouth in which over 40 per cent of the plants were affected by this disease. On many of these plants the leaves were so badly affected as to considerably reduce the yield. During the summer of 1916, no cucumbers were grown in any of the fields above mentioned and although a sharp watch was maintained, the disease was not found in the vicinity of Plymouth. However, while making a survey of the cucumber fields in northern Indiana about the middle of September, 1916, the disease was again found in fields around Hamlet, Lapaz, and North Liberty. While the disease was not sufficiently widespread to prove a serious economic factor in the growing of cucumbers, observations indicate that in those spots of the field in which it occurred the leaves were so badly injured as to reduce appreciably the yield for pickles.

SYMPTOMS OF THE DISEASE

The disease first appears in the form of small yellowish spots on the leaf, usually 0.5 to 1 mm. in diameter. The spots are soon visible on both the upper and lower surfaces of the leaf (Pl. 21, A, B). The

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center of the upper surface of the spots soon changes to a light yellowish brown with a reddish brown border. Around this border for 1 to 2 mm. the leaf becomes light green. In some cases the spots may be almost white. The color depends apparently on the amount of sunlight as well as on the humidity. On the lower surface the spots remain lighter colored and do not develop the dark border. Mature lesions are of two distinct types; one a small spot, the other a large mottled spot, depending on the abundance of infection and on weather conditions. The small spots are produced most abundantly when infection is followed by dry weather. They range from 0.2 mm. to 3 or 4 mm. in diameter, the majority being 1 to 2 mm. (Pl. 21, A, B). At first circular in outline, they may become irregular or markedly angular, being limited by the veins of the leaf. As already mentioned, there may be considerable variation in the color of the spots, in some cases the reddish brown border being almost entirely absent.

When infection is followed by moist weather, and especially if several spores germinate near each other in a large drop of water, a different type of lesion is produced. These lesions are larger, ranging from 4 to 15 mm. in diameter (Pl. 22, A, B). They vary from white to light brown, even in the same spot, with reddish-brown areas along the veins. This gives them a characteristic mottled appearance. These large spots may originate in various ways. In some cases the infection appears over the large spot all at once; in others it first appears as several small lesions which later unite; while in others the infection may later extend outward from a central point. In the last-named case there may be a dark-brown center of 3 to 5 mm. with a lighter area around it. On the lower surface of the leaf the mottled appearance is much less distinct, the spots usually being light yellowish with occasionally a slight tinge of brown. During hard rains the centers of these spots may be broken out, leaving holes in the leaves. In severe cases the leaf may be so badly infected that it turns yellow or brown and dies.

The writer has not seen infection on stems, petioles, or fruit in the field, but occasional longitudinal lesions on stems and petioles have been produced by artificial inoculation in the greenhouse. These spots are slightly sunken, 0.5 to 1 mm. wide by 3 to 5 mm. long, with a very light yellowish-brown center and darker border.

ISOLATION OF THE CAUSAL ORGANISM

Material collected both in 1915 and 1916 was invariably found to have associated with the spots a fungus belonging to the Dematiaceae-Dictyosporae group of the Hyphomycetes.

Since the fungus produces rather large spores on a surface mycelium, spore dilution in plate cultures was found to be the easiest method of obtaining it in pure culture. Young spots free from dirt on which there was abundant spore formation were inverted under a binocular micro-

scope and a few spores transferred by means of a sterile, moistened scalpel to string-bean agar. Dilution plate cultures were then made. As soon as the spores had germinated, as shown by examination under a microscope, single spores were transferred to other agar plates. When these cultures produced spores, single-spore isolations were again made to make doubly sure they were free from contamination. In a few cases the fungus was isolated by the tissue-culture method from leaves. Infected leaves were sterilized by washing for one-half to three minutes in a 1 to 1,000 solution of mercuric chlorid or in 95 per cent alcohol. They were then thoroughly washed with distilled water and plated in string-bean or potato agar. In some cases these cultures were overrun by various mold fungi, but in others a fungus identical in all respects with that obtained from the spore dilutions was isolated. The fungus was isolated from material collected in 1916 at Lapaz and Hamlet, Ind. These isolations were labeled as strains 1 and 2, respectively. A tissue culture isolation from Lapaz was labeled "strain 3." Cultures from two of the mold fungi were retained and used for later inoculations.

INOCULATION EXPERIMENTS

Inoculation tests were made with cultures from the strains mentioned above as well as with spores taken directly from diseased cucumber leaves. All strains, as well as the fresh spores, developed abundant infection. Inoculations made with the other fungi isolated from the diseased spots failed to develop infection. Most of the inoculations were made in the greenhouse, only one series being made in the field. In both cases the inoculations were successful.

One series of inoculations made on September 13, 1916, will be described in detail as being typical of the manner in which all were made. Spores of strains 1, 2, and 3 were taken from string-bean-agar cultures 15 days old. Contemporaneous germination tests on slides showed that the spores were viable. Inoculations were made with suspensions of these spores in distilled water, tap water, and beef bouillon. The spores were applied to both upper and lower surfaces of the leaves by spraying the suspension from an atomizer or by dropping it from a pipette. In spraying with the atomizer some of the spores doubtless occasionally reached both surfaces. Four plants were used with each strain for each different method of inoculation, 12 plants being sprayed with water and 6 with bouillon as controls. One-half of the plants were left in the dry air of the greenhouse. The others were placed for 24 hours in a glass culture chamber so arranged that fresh air was admitted from below, and after this period they were also kept in the greenhouse. The results on September 17, four days later, are summarized in Table I.

TABLE I.—*Infection showing on September 17, 1916, on cucumbers inoculated on September 13*

Manner of inoculation.	Infection on plants kept in—	
	Culture chamber for 24 hours.	Greenhouse from beginning.
Leaves sprayed on upper surface	Fine spotting (0.2 to 0.75 mm. in diameter).	No infection
Leaves sprayed on lower surface	.....do.....	Do.
Leaves with drops on upper surface.	Fine spotting in groups.....	Do.
Leaves with drops on lower surface.	.....do.....	Do.
Controls.....	No infection.....	Do.

Infection occurred equally well with all the strains used and regardless of whether the spores were applied in distilled water, tap water, or beef bouillon.

An inspection of Table I shows the following points: The infection appeared on both surfaces regardless of which surface had been inoculated. Infection appeared only on those plants kept in a moist atmosphere for some hours after inoculation. As might be expected, those leaves that were sprayed with the atomizer showed a fine spotting over more or less of the entire surface, while on those inoculated with large drops of liquid the spots occurred in groups. In this series of inoculations the spots did not continue to enlarge and spores were produced on only a few of the lesions. The fungus was reisolated both from the spots that produced spores and from those that did not.

The question then arose as to the cause of the failure to produce the large mottled spots with normal spore formation so common in the field. The plants had been kept in the greenhouse at a rather high temperature and low humidity. Accordingly another series of inoculations was started in which these factors were varied. It was found that under conditions of fairly high humidity and of moderate temperature the characteristic mottled spots were produced with abundant spore formation on the lower surface of the leaves. Under conditions of excessive humidity and partial shade, spore formation occurred on both surfaces of the leaf, a phenomenon very rare in nature.

Other inoculations, in which the writer used mycelium in comparison with suspensions of spores, have shown that infection occurs equally well in either case.

On September 15, 1916, a series of inoculations was made on cucumber, gourd, and squash (*Cucurbita* spp.) with a suspension of spores in water. The plants were placed in the culture chamber for 24 hours and then kept in the greenhouse. Infection appeared on the cucumbers, but not on the gourds or squashes. Later, these experiments were repeated, the

plants being kept under conditions more favorable for infection. In this case infection occurred on all the inoculated plants, cucumbers, egg and pear-shaped gourds, crookneck and White Bush Scalloped squashes (Pl. 23, A. B). These exepriments have been repeated in the field with similar results.

Repeated attempts have been made to obtain infection on other parts of the host than the leaves. In the case of cucumbers only, an occasional infection on stem and petiole has resulted when the plants were kept continuously in the culture chamber.

The various inoculation experiments have afforded abundant opportunity for observing the effect of age on the susceptibility of leaves to infection. Infection may occur on the youngest unfolding leaves, but is much more abundant on the older leaves. The spots develop more normally on the older leaves, and spore formation occurs earlier and more abundantly.

Through the inoculation experiments outlined above, the parasitism of this fungus to the leaves of cucumbers, and certain varieties of gourds and squashes would seem to be clearly established. Successful inoculations were made on the following four varieties of cucumbers: Arlington White Spine, Nichol's Medium Green, Heinz Muscatine, and Davis Perfect.

TAXONOMY AND DESCRIPTION OF THE FUNGUS

This fungus belongs in the family Dematiaceae of the Hyphomycetes. The dark, muriform, subglobose conidia place it in the group Dictyosporae. Careful consideration of the fungus, both in the field and in artificial culture has led the writer to place it in the genus Stemphylium.[1]

The following is a brief description of the fungus:[2]

Stemphylium cucurbitacearum, n. sp.

The fungus produces spots ranging from 0.2 to 15 mm. in diameter upon leaves, rarely stems and petioles. The smaller spots vary from circular to angular in outline, with a light yellowish brown center and a darker reddish brown border, rarely white, on the upper surface; the spots are lighter on the lower surface. The larger spots vary from white to light brown, with reddish brown areas along the veins, giving a mottled appearance.

The mycelium is hyalin to light brown, vacuolate, septate, branched, growing readily on various culture media. Diameter, 2.5 to 10 μ. Fusion of the hyphæ is quite common.

[1] A specimen of this fungus was submitted to Dr. W. G. Farlow who verified the writer's conclusions in regard to its systematic position.

[2] **Stemphylium cucurbitacearum,** sp. nov.—Maculis in foliis, raro caulibus, 0.2 to 15 mm. in diameter; maculis minoribus orbicularibus vel angularibus, ad superficiem centro pallido-luteo-brunneis, rufo-brunneo-marginatis raro albidis, infra albidis; maculis largioribus albidis vel laete-brunneis per venas rufis-fuscis; mycelio hyalino vel laete-brunneis; septato, ramoso; sporophoris hyalinis vel laete-brunneis 1–5 septatis, 10–30 × 7–12 μ, cellulis singulis deinde globosis; sporidiis muriformibus, subglobosis, atro-brunneis 25–50 μ diameter, e cellulis 5–20, 10–18 μ diameter, hypophyllis, in apice sporophororum singulis.

Habitat in foliis vivis, raro caulis, *Cucumeris sativi* et *Cucurbitae peponis*, Plymouth, Lapaz, Lakeville, Hamlet, et North Liberty, Indiana; et Bowling Green, Ohio, America Borealis.

Sporophores arise singly on the lower surface of the leaf as branches of the mycelium, hyalin to light brown, 1- to 5-septate, 10 to 30 by 7 to 12 μ, the individual cells eventually becoming globose and easily breaking apart, bearing a single spore at the apex.

The spores are nearly globose, dark brown, muriform, 25 to 50 μ in diameter, composed of 5 to 20 cells each of which are 10 to 18 μ in diameter, easily breaking away from the sporophores. They develop abundantly on the lower surface of leaves and on several of the ordinary culture media.

Habitat: Leaves, rarely stems and petioles, of *Cucumis sativus* and *Cucurbita pepo* (gourds and summer squash). The type specimen was collected from a cucumber field, near Plymouth, Ind., on September 27, 1915. Specimens were also collected from Lapaz and Lakeville, Ind., in September, 1915, and Bowling Green, Ohio, in October, 1915 (W. W. Gilbert); Hamlet and North Liberty, Ind., in September, 1916.

The type specimen has been deposited in the herbarium of the United States Department of Agriculture, Washington, D. C. Duplicates of this type material have been deposited in the herbaria of the New York Botanical Garden, New York, the Harvard Cryptogamic Laboratory, Cambridge, Mass., and the Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Ind.

CULTURAL CHARACTERS OF THE FUNGUS

The fungus has been grown on the following media: Corn-meal agar, beef agar, Lima bean agar, oat agar, potato-glucose agar, potato plugs, cucumber-stem agar, string-bean agar, and beef bouillon. On standard beef agar and in bouillon a limited growth with slight spore production occurs. On potato-glucose agar and string-bean agar abundant growth with abundant spore production takes place. The growth on the other media tried is intermediate between these two.

The growth on one series of string-bean-agar cultures will be given in detail as a typical example. These transfers were made on November 9, 1916, with spores from a 15-day-old culture.

November 11, 1916.—Growth was visible around point of inoculation as light radiating strands of mycelium.

November 14, 1916.—Growth around point of inoculation was rather granular for 2½ to 3 mm. in diameter. Light radiating growth around this was 6 to 8 mm. in diameter. Examination with a binocular microscope showed that the granular appearance was due to the beginning of spore formation. A few drops of liquid were present on mycelium in some tubes, owing to exudation.

November 17, 1916.—Growth was 12 to 14 mm. in diameter. A thin layer of brown spores 8 to 10 mm. in diameter, with granular-appearing mycelium, surrounded this. Around portions of the margin there was a narrow light flocculent growth where spore formation had not yet begun.

November 22, 1916.—Growth was 16 to 20 mm. in diameter. The surface was almost entirely covered with a heavy dark-brown or nearly black mass of spores. The spore formation was less abundant at the edge, or in places was absent. A few white tufts of mycelium 1 to 2 mm. in diameter had appeared over the spores.

December 1, 1916.—Heavy dark spore masses appeared in all the tubes, partly covered by a white fluffy growth of mycelium.

At first this fluffy growth of mycelium following heavy spore production was rather puzzling. Careful reisolation of all strains showed that it was not due to contamination. Further observations have shown that it arises from germination of the spores in the cultures.

RELATION TO LIGHT.—Sixteen tubes each of string-bean and potato-glucose agar were inoculated with two strains of the fungus, and one-half were kept in the dark, while the others were exposed to the ordinary light of the laboratory. No difference could be observed in their growth.

RELATION TO MOISTURE.—Growth was usually slower on media that had dried somewhat on the surface. It was also slower in a dry atmosphere than when the tubes were placed in a moist atmosphere under a bell jar or in the culture chamber. In the latter cases the mycelium exuded copious drops of liquid. Spore formation was usually retarded in a moist atmosphere.

RELATION TO TEMPERATURE.—Cultures on string-bean and potato-glucose agar were exposed to various temperatures. For room temperature, 18° to 23° C., the regular laboratory was used. For temperatures above this the cultures were kept in the incubator. For temperatures below room temperature two different cold rooms were employed. The temperature in these cold rooms was not entirely uniform, but did not vary over 3° or 4°. The optimum temperature for development and spore production was found to be from 18° to 25°. Above 30° and below 15° growth was very scant, and only a few spores were produced. This low optimum may explain the writer's failure to find infection in the cucumber fields around Plymouth during the summer of 1916 before the cooler weather of September had arrived. During midsummer the average temperature was much above the maximum for the growth of this fungus. The writer has had no success in securing infections where the temperature has been high continuously at the time of inoculation.

SPORE FORMATION

In artificial cultures spore formation may be so abundant that the contents of the mycelium are almost entirely used up. The conidiophore begins as an outgrowth of a mycelial cell (fig. 1, A, B, C). It is at first nonseptate, but a septum is soon laid down near the mycelium, and as growth proceeds, additional septa are formed, in extreme cases as many as eight being produced (fig. 1, C–F). The end cell, or in some cases the cell next to the end, now divides by a longitudinal septum (fig. 1, G–I). Other longitudinal divisions follow rapidly, and in a short time a globular head consisting of from 5 to 20 cells or in extreme cases even more is produced (Pl. 24, B, C; and fig. 1, M, N). Meanwhile, as the longitudinal divisions progress, the cells gradually assume a dark-brown color, caused by a pigment in the cell wall.

The cells of the conidiophore, which at first are compressed tightly together, gradually become globose and loosely attached to each other and to the spore (fig. 1, M, N).

SPORE GERMINATION

To observe spore germination, Van Tieghem cells and slides supported in petri dishes were used. In the latter case drops of liquid containing the spores were placed directly on the slide. The spores were scraped from the surface of the culture or leaf and filtered through fine silk bolting cloth to separate them from each other, and they were then dropped on the slides. With the Van Tieghem cells the spores were transferred directly to the covers from cultures.

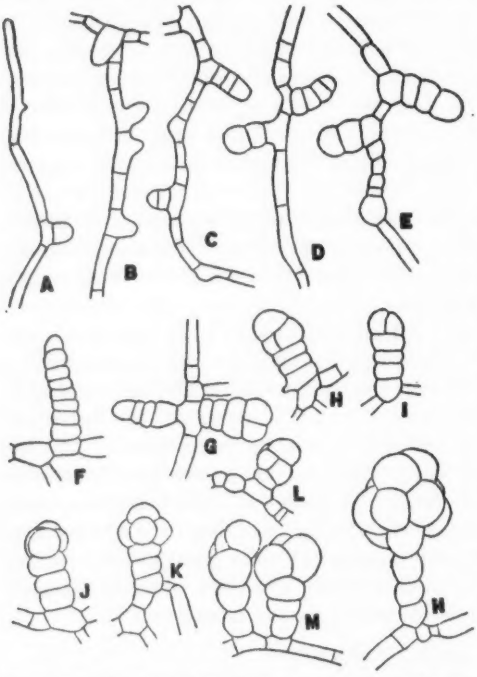

FIG. 1.—Spore formation of *Stemphylium cucurbitacearum* (Material taken from string-bean-agar cultures 12 days old. ×1,050): *A*, *B*, earliest stages of spore formation, showing origin of conidiophores on mycelium; *C–F*, early stages in spore formation, showing the transverse divisions of the conidiophores; *G–N*, various stages in the maturation of the spores.

The first signs of germination consist in a slight swelling of the spore owing to increased turgidity. In from three to six hours some of the cells were observed to have a slight protuberance, the beginning of the germ tube (fig. 2, A, F). Two hours later the germ tube was found to have pushed out 5 to 10 mm. (fig. 2, B, G). In beef bouillon or other nutrient bouillon the growth is fairly rapid from now on. In fig. 2, A–E, is shown a spore in various stages of germination. Not all of the cells germinate, but in the case of large spores the writer has observed as many as a dozen germ tubes. Only one germ tube is produced from a single cell. The germ tubes branch profusely and soon become septate (Pl. 24, A; fig. 2, E, H). Within 72 hours they have frequently become a tangled mass of threads. Fusions of the hyphæ are quite common (fig. 3).

In water, germination is slower, and growth soon ceases. Frequently vesicular, highly vacuolate, thin-walled bodies are produced (fig. 2, I, J). These may break away, or a germ tube may grow from them while still attached to the original germ tube. They probably have no significance in the life history of the organism.

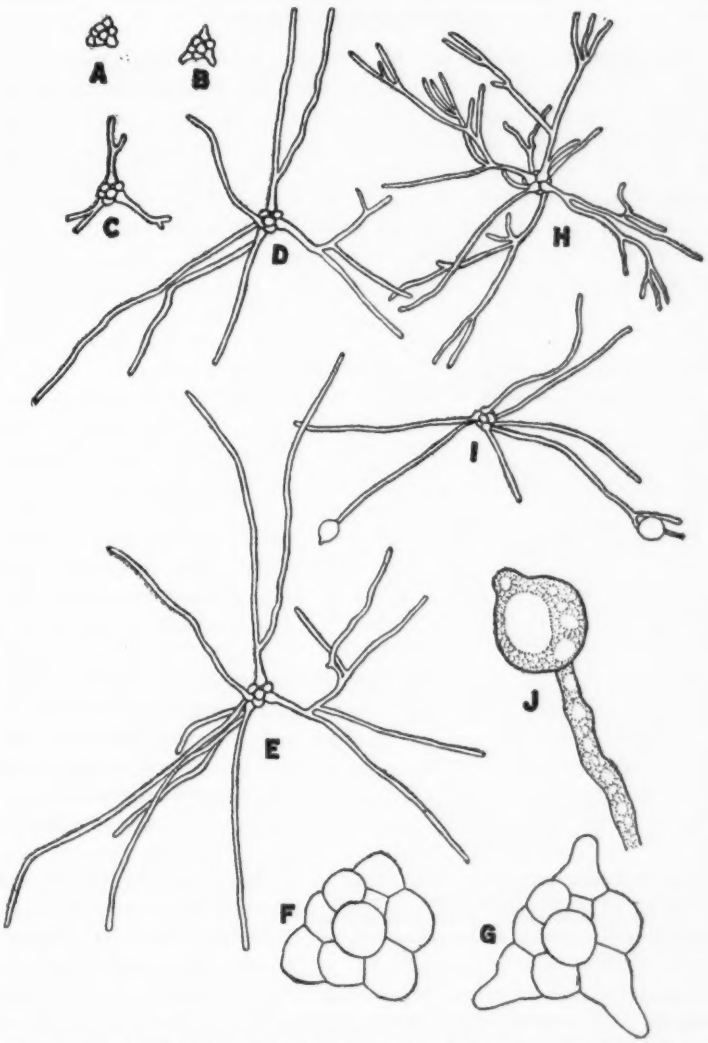

FIG. 2.—Spore germination of *Stemphylium cucurbitacearum: A–E*, various stages in the germination of a single spore in nutrient bouillon. *A*, 6 hours; *B*, 8 hours; *C*, 12 hours; *D*, 24 hours; *E*, 36 hours. ×105. *F–G*, same as *A–B*, but more highly magnified, showing origin of germ tubes. ×1,190. *H*, spore germinated in nutrient bouillon after 48 hours. ×105. *I*, spore germinated in distilled water after 48 hours, showing thin-walled vesicular bodies. ×105. *J*, portion of the germ tube shown in *I* more highly magnified. ×1,190.

The maturity of the spore has nothing to do with its capacity for germination. In Plate 24, A, is shown a germinating spore which has not yet begun to turn brown. The writer has secured germination from the earliest stages of spore formation.

LIFE-HISTORY STUDIES

The inoculation period for this fungus is usually from three to five days. Infection may occur either on the upper or lower surface of the leaves. In a few cases the writer has observed germ tubes entering through the opening of the stomata. Whether this is the only method of infection was not determined. For observing this phenomenon pieces of inoculated tissue were fixed and stained with eosin or sectioned and stained with Fleming's triple stain. The mycelium penetrates the tissues of the leaf, causing them to collapse somewhat. In from 8 to 12 days spore formation is found to be taking place on the lower surface of the leaf. The conidiophores arise from mycelium growing on the surface.

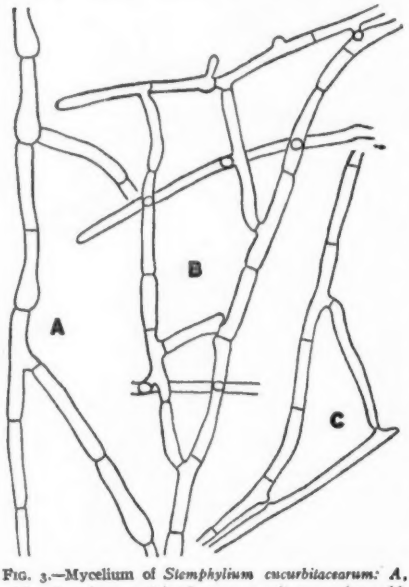

FIG. 3.—Mycelium of *Stemphylium cucurbitacearum*: *A*, Mycelium from a string-bean-agar culture 12 days old. ×1,050. *B–C*, Mycelium from spores germinated in nutrient bouillon, 72 hours old, showing fusions of the hyphæ. ×1,190.

The fungus appears to overwinter as mycelium in the tissues of the host. Whether the spores may also live over a winter has not been definitely determined. Viable spores were obtained from both cultures and leaves that were exposed outside over a winter, but it was impossible to determine whether these spores had overwintered or had been produced in the spring. Spores taken from culture media and placed outside in paper packets failed to germinate in the spring.

The spores may be scattered by wind, rain, insects, animals, or garden tools. When slightly wetted, the spores have a tendency to stick together, owing apparently to surface tension phenomena. This would allow them to be easily carried by insects or animals. The spores may be easily spattered about by raindrops. To test this point, the writer inoculated the upper leaves of three plants; and as soon as spore formation occurred, the plants were sprayed with water. In a few days infection was general on the lower leaves, while healthy plants sprayed as controls remained free from disease. The spores may also be carried short distances at least by strong winds. The writer has caught spores on the moistened slides several inches from leaves held in the current of an electric fan.

CONTROL OF THE FUNGUS

Certain preliminary experiments give promise that this disease may be easily controlled by spraying the leaves with Bordeaux mixture.

In the laboratory the slides were sprayed in the manner outlined by Wallace, Blodgett, and Hesler.[1] The method was as follows: One end of a glass slide was sprayed with the spray mixture, and after drying, the slide was supported on glass slips in a petri dish. A suspension of spores in water evenly distributed by filtering through silk bolting cloth was placed on both the sprayed and unsprayed ends of the slide. In Table II is given the percentage of germination from the various treatments.

TABLE II.—*Percentage of the spore germination of Stemphylium cucurbitacearum on slides after spraying with Bordeaux mixture*

Strength of Bordeaux mixture.	April 30.		May 10.		May 12.		May 15.		Average.	
	Sprayed.	Control.	Sprayed.	Control.	Sprayed.	Control.	Sprayed.	Control.	Sprayed.	Control.
1–2–50....	25	63	3	77	14	70	19	85	15	74
2–4–50....	12	63	3	75	4	62	10	90	7	72
3–6–50....	3	66	Tr.	79	1	66	4	80	2	73
4–6–50....	Tr.	63	Tr.	76	Tr.	63	Tr.	82	Tr.	71
5–5–50....	1	61	0	70	Tr.	70	Tr.	82	Tr.	71

In each case the figures given represent the average of three slides. Germination is recorded regardless of whether the germ tube was long or short. In many cases, especially where the higher strengths of Bordeaux mixture were used, the germ tubes were so short that infection could hardly have taken place. For example, on the slides sprayed with Bordeaux mixture 1–2–50 the germ tube averaged about 40 μ, while on the controls they were several hundred microns in length. Consequently the figures given in Table II may underestimate rather than overestimate the value of the spray mixture, provided the leaves are thoroughly and evenly coated. To judge from the results given in Table II, Bordeaux mixture 3–6–50 would appear to be entirely efficient against this disease.

To test this point further, four plants were sprayed with Bordeaux mixture 2–4–50 and 3–6–50, respectively. The next day these plants were inoculated with a suspension of spores from a potato-glucose-agar culture. Four plants sprayed with water were inoculated as controls. Two small spots developed on the plants sprayed with Bordeaux mixture 2–4–50, the other plants sprayed with the mixture remaining healthy. The control plants developed numerous infections.

[1] WALLACE, Ettett, BLODGETT, F. M., and HESLER, L. R. STUDIES OF THE FUNGICIDAL VALUE OF LIME-SULPHUR PREPARATIONS. N. Y. Cornell Agr. Exp. Sta. Bul. 290, p. 167–174. 1911.

In addition to spraying, certain sanitary measures should be adopted. Diseased vines should be destroyed, if possible, in the fall. Crop rotation should be practiced. Vines should not be disturbed when wet with dew or rain.

SUMMARY

During the summers of 1915 and 1916 the attention of the writer was called to a peculiar leafspot on cucumber that was doing more or less damage to cucumber fields in the vicinity of Plymouth, Ind., and Bowling Green, Ohio. The spots vary in diameter from 0.2 to 15 mm. The small spots, ranging from 0.2 to 3 or 4 mm. in diameter, may be circular or angular in outline. The center is light yellowish brown, surrounded by a reddish-brown border; rarely, the spot is nearly white. The larger spots are usually nearly white or tinged with brown, with reddish-brown areas along the veins and frequently with brownish centers, giving the spots a mottled appearance. From these spots a fungus belonging to the genus Stemphylium has been isolated. The parasitism of this fungus to the leaves of cucumbers, gourds, and squashes has been repeatedly demonstrated by successful inoculations. Four varieties of cucumber, two of gourd, and two of squash have been successfully inoculated. So far as ascertained, no data regarding this disease has been published hitherto, and the causal organism is described as a new species under the name "*Stemphylium cucurbitacearum.*"

Cultural work with this fungus has shown that high temperatures and a dry atmosphere are unfavorable to its development. Consequently serious trouble may be expected only in cool weather, especially when combined with abundant moisture.

The fungus lives over the winter in or on the diseased vines. The spores are disseminated by wind, rain, insects, etc.

Preliminary experiments have given promise that this disease may be controlled by Bordeaux mixture. In addition to this, sanitary measures such as the destruction of diseased vines and crop rotation, as well as the avoidance of work among plants when wet, should be practiced.

VAN GOGH

A RETROSPECTIVE

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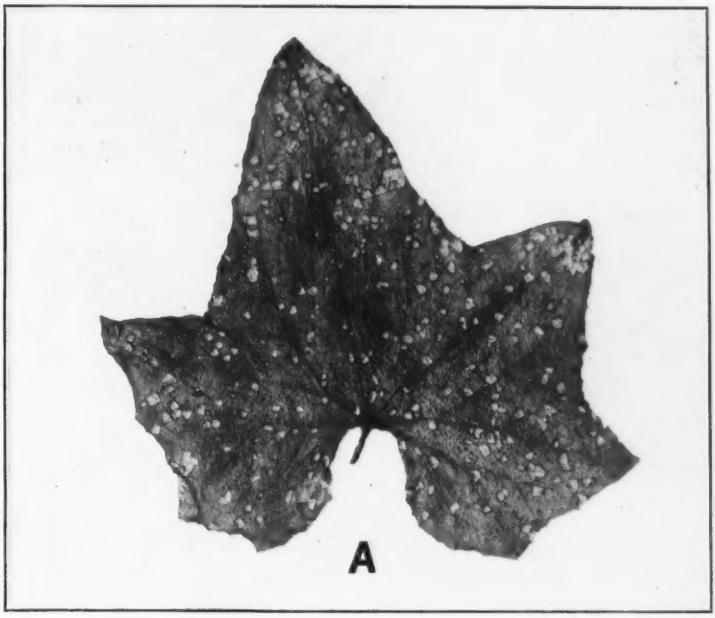
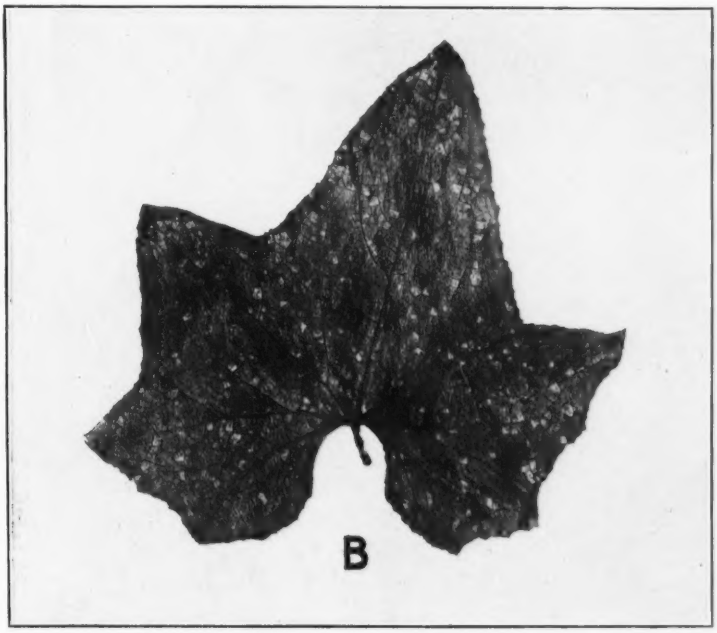
PLATE 21

Stemphylium cucurbitacearum

A.—Small spots on upper surface of leaf with a few larger spots formed by coalescence of small spots.

B.—Same leaf as shown in A, but lower surface view.


A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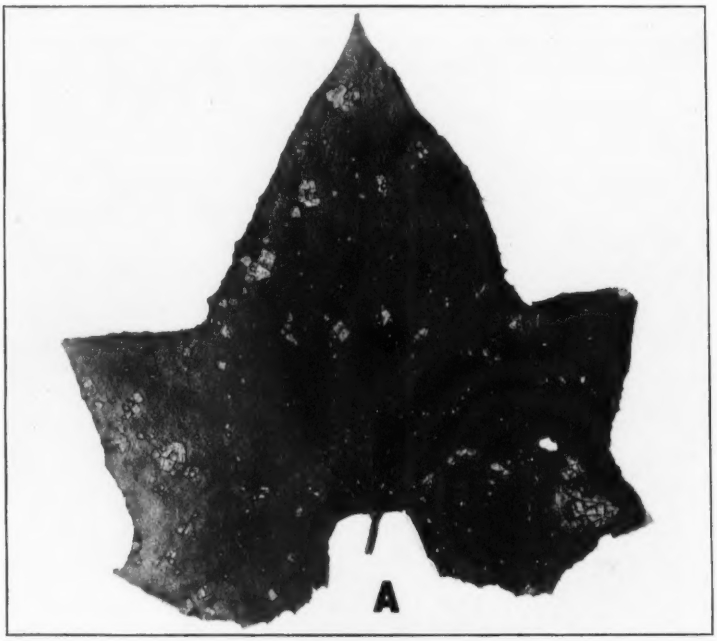
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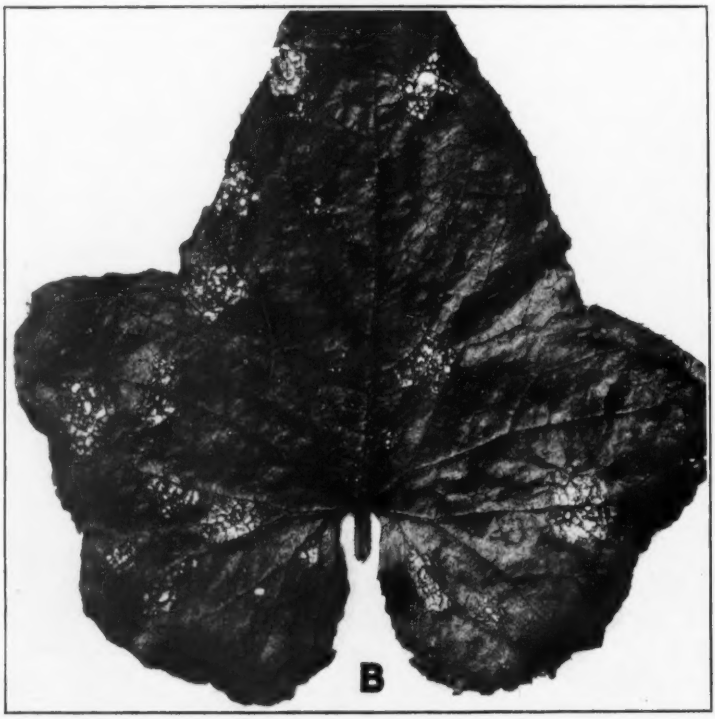

B

PLATE 22

Stemphylium cucurbitacearum

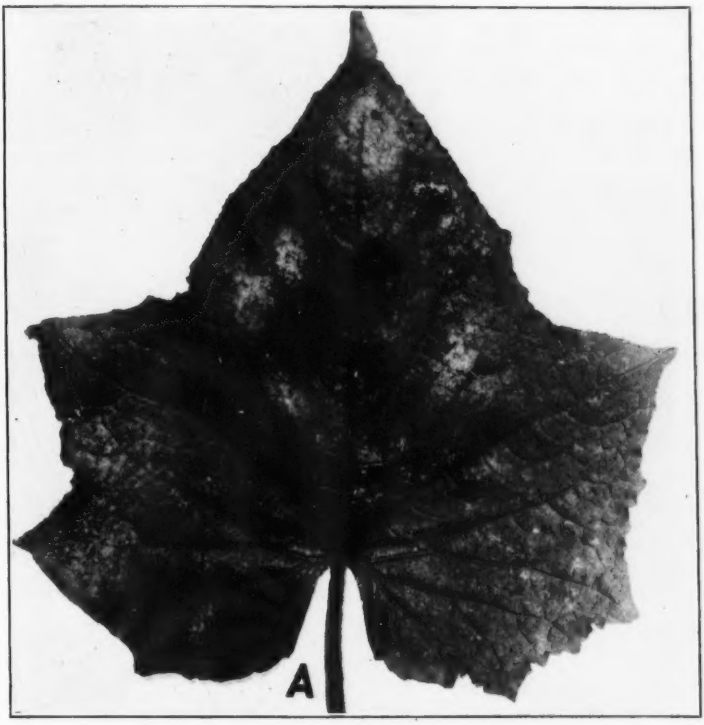
A.—Large and small spots on upper surface of cucumber leaf.

B.—Large spots on cucumber leaf showing brown centers surrounded by lighter area.

PLATE 23

A.—Early stage of the Stemphylium leafspot on cucumber from artificial inoculation, showing the formation of the large mottled spots.

B.—Early stage of the Stemphylium leafspot on gourd from artificial inoculation, showing very fine spots in groups.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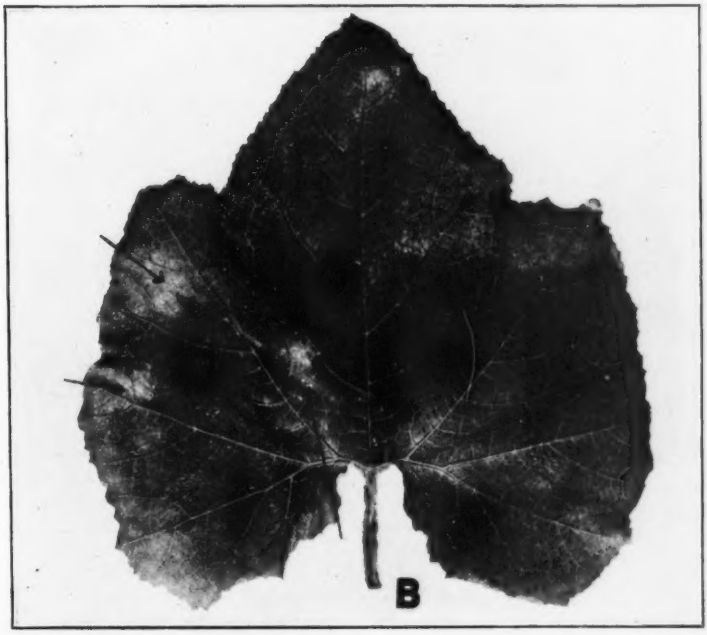
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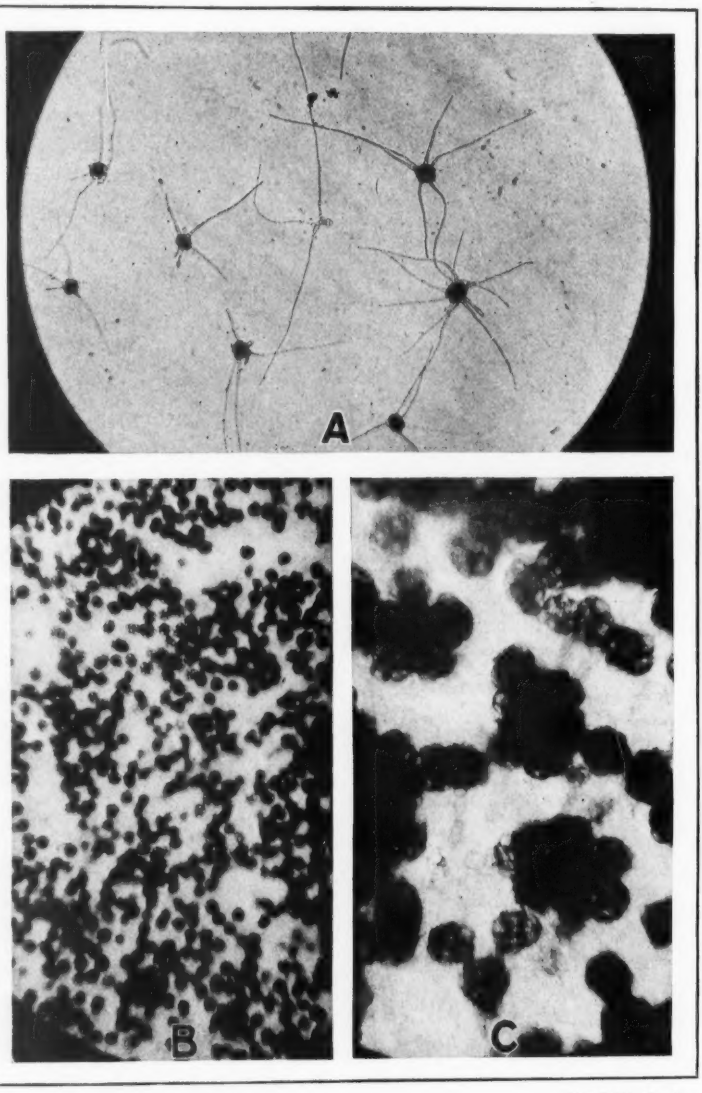
A
B
C

PLATE 24

Spore formation and germination of *Stemphylium cucurbitacearum*

A.—Spores germinating in tap water. ×95.
B.—Spore formation in string-bean-agar culture 15 days old. ×60.
C.—Portion of B more enlarged. ×225.